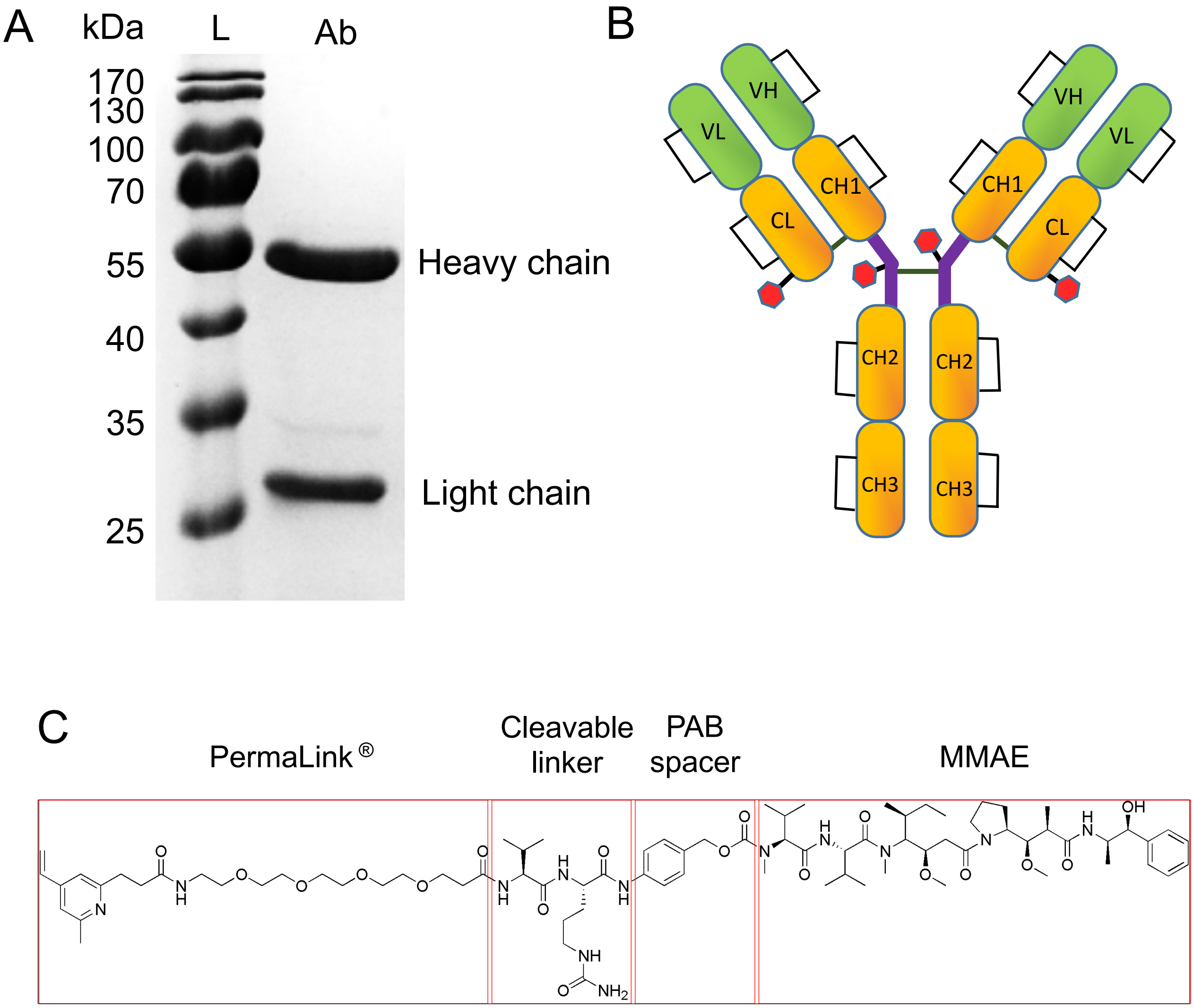
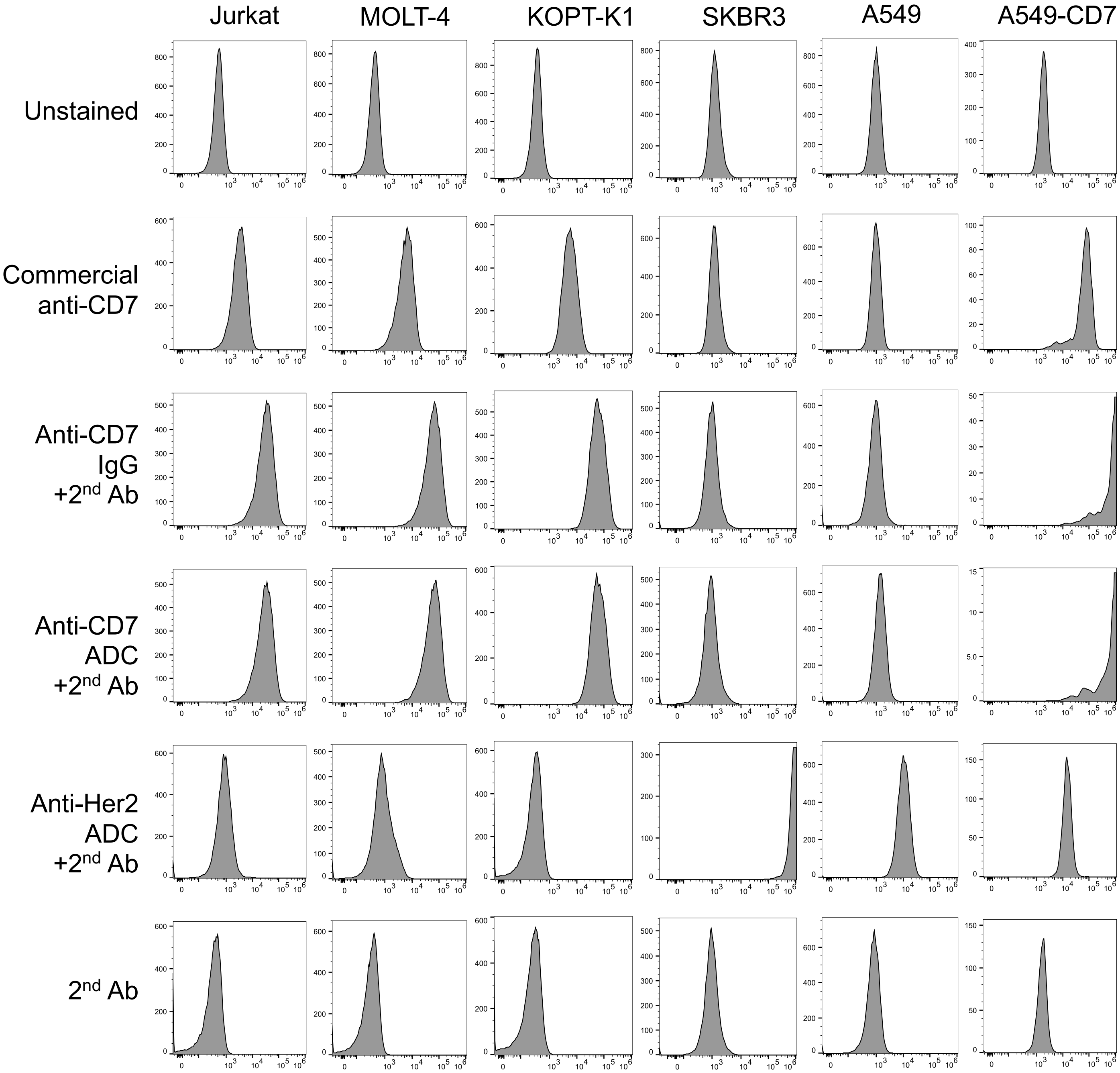
Supplementary Figure 1



Supplementary Figure 1. Analysis of anti-CD7 Ab and structure of anti-CD7-PermaLink-MMAE ADC.
A. SDS-PAGE analysis of purified anti-CD7. L, protein ladder; Ab, purified anti-CD7 sample. B. Illustration of anti-CD7-MMAE ADC. The conjugated drugs are shown as red hexagons. C. The structure of the payload of anti-CD7 ADC consisting of the cytotoxic drug monomethyl auristatin E (MMAE), a p-aminobenzyl (PAB) spacer, a valine-citrulline cleavable linker and PermaLink® conjugation chemistry.

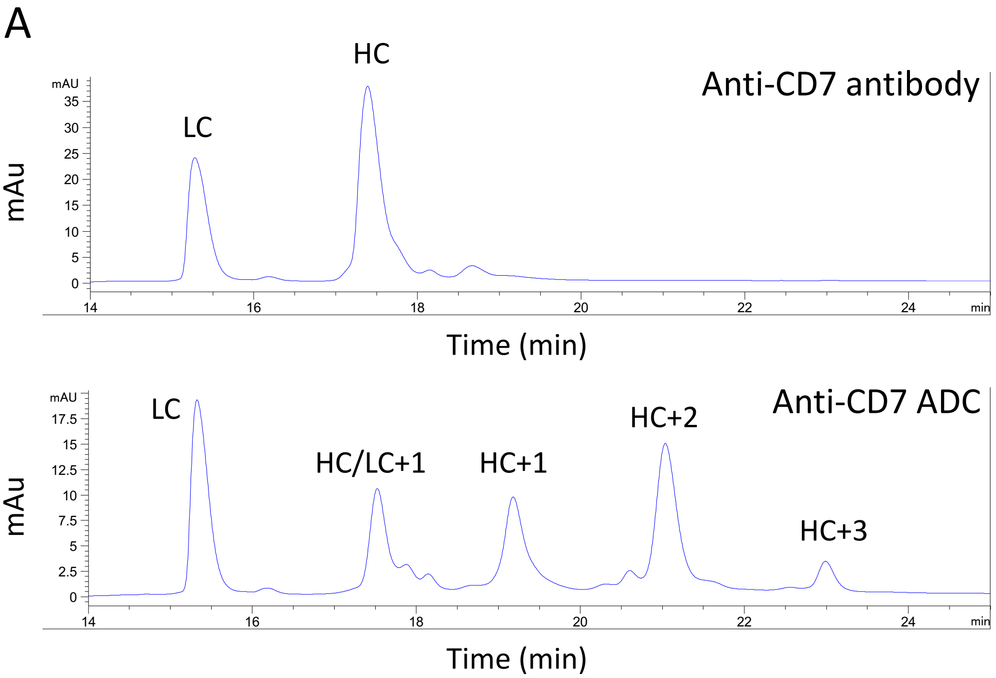
Supplementary Figure 2



Supplementary Figure 2. Anti-CD7, anti-Her2 and anti-CD7-MMAE antibody binding to CD7 and Her2 on cell surface

Cells tested in the *in vitro* ADC cytotoxicity experiments were analyzed for cell surface expression of CD7 and Her2 using commercial anti-CD7 (FITC labeled), anti-CD7 IgG, anti-CD7-MMAE ADC and anti-Her2-MMAE ADC. Alexa Fluor 488 labeled goat-anti-human IgG was used for the secondary staining of samples stained with anti-CD7 IgG, anti-CD7-MMAE ADC and anti-Her2-MMAE ADC.

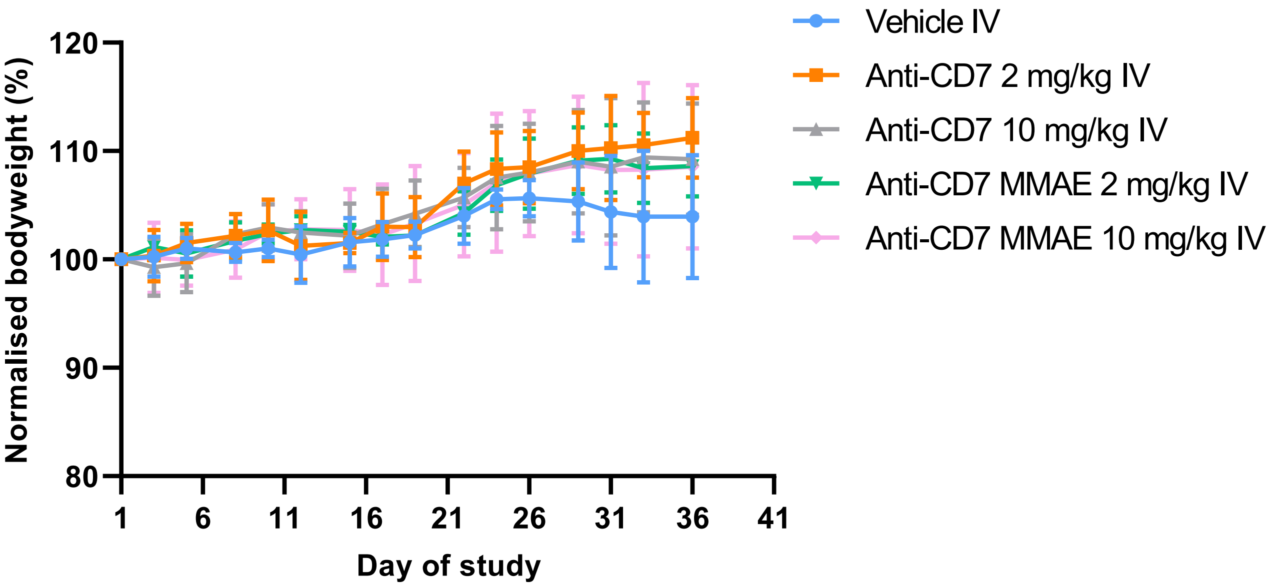
Supplementary Figure 3



Supplementary Figure 3. PLRP analysis of the anti-CD7 antibody and ADC.

The unconjugated anti-CD7 antibody and drug conjugated anti-CD7 ADC were analysed using PLRP as described in the materials and methods section. Peaks exhibiting a different drug loading are labelled with numbers 0-3. HC: heavy chain; LC: light chain. The DAR was estimated to be 2.6

Supplementary Figure 4



Supplementary Figure 4. Normalized bodyweight of male athymic nude mice dosed with anti-CD7 or anti-CD7-MMAE.
Values shown are mean ±SEM; n=5 for all groups.

Supplementary Figure 5. Sequence of anti-CD7-expressing vectors

The VH and VL of anti-CD7 were amplified using PCR from the vector pET26b anti-CD7 scFv and cloned into VHExpress and VKExpress vectors.

Panel A. The DNA sequence and the translated amino acid sequence of anti-CD7 single chain Fv.

Panel B. The sequence of pET26b anti-CD7 scFv plasmid. The DNA sequence and the translated amino acid sequence of anti-CD7 scFv are shown in red.

Panel C. The sequence of VKExpress anti-CD7 VL plasmid. The DNA sequence and the translated amino acid sequence of anti-CD7 VL are shown in red. The exon sequence of CL and the translated amino acid sequence are shown in blue.

Panel D. The sequence of VHExpress anti-CD7 VH plasmid. The DNA sequence and the translated amino acid sequence of anti-CD7 VH are shown in red. The exon sequence of CH and the translated amino acid sequence are shown in blue.

A. Sequence of anti-CD7 scFv

```
atgaaatacctattgcctacggcagccgctggattgttattactcgcggcccagccggcc
 M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A 
atggcggcctacaaagatatccagatgacacagactacatcctccctgtctgcctctctg
 M  A  A  Y  K  D  I  Q  M  T  Q  T  T  S  S  L  S  A  S  L 
ggagacagagtcaccatcagttgcagtgcaagtcagggcattagcaattatttaaactgg
 G  D  R  V  T  I  S  C  S  A  S  Q  G  I  S  N  Y  L  N  W 
tatcagcagaaaccagatggaactgttaaactcctgatctattacacatcaagtttacac
 Y  Q  Q  K  P  D  G  T  V  K  L  L  I  Y  Y  T  S  S  L  H 
tcaggagtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatc
 S  G  V  P  S  R  F  S  G  S  G  S  G  T  D  Y  S  L  T  I 
agcaacctggaacctgaagatattgccacttattattgtcagcagtatagcaagcttccg
 S  N  L  E  P  E  D  I  A  T  Y  Y  C  Q  Q  Y  S  K  L  P 
tacacgttcggaggggggaccaagctggaaataaaacgtggtggtggtggttctggtggt
 Y  T  F  G  G  G  T  K  L  E  I  K  R  G  G  G  G  S  G  G 
ggtggttctggcggcggcggctccggtggtggtggatccgaggtgcaactggtggagtct
 G  G  S  G  G  G  G  S  G  G  G  G  S  E  V  Q  L  V  E  S 
gggggaggcttagtgaagcctgggggtccctgaaactctcctgtgcagcctctggactc
 G  G  G  L  V  K  P  G  G  S  L  K  L  S  C  A  A  S  G  L 
actttcagtagctatgccatgtcttgggttcgccagactccagagaagaggctggagtgg
 T  F  S  S  Y  A  M  S  W  V  R  Q  T  P  E  K  R  L  E  W 
gtcgcatccattagtagtggtggtttcacctactatccagacagtgtgaagggccgattc
 V  A  S  I  S  S  G  G  F  T  Y  Y  P  D  S  V  K  G  R  F 
accatctccagagataatgccaggaacatcctgtatctgcaaatgagcagtctgaggtct
 T  I  S  R  D  N  A  R  N  I  L  Y  L  Q  M  S  S  L  R  S 
gaggacacggccatgtattactgtgcaagagacgaggtacgggggtacctcgatgtctgg
 E  D  T  A  M  Y  Y  C  A  R  D  E  V  R  G  Y  L  D  V  W 
ggcgcagggaccacggtcaccgtttcctcggcctcgggggccgatcaccatcatcaccat
 G  A  G  T  T  V  T  V  S  S  A  S  G  A  D  H  H  H  H  H 
cattgctagtaa
 H  C  -  - 
```

B. Sequence of pET26b anti-CD7 scFv plasmid

```
         10        20        30        40        50        60
 ATCCGGATATAGTTCCTCCTTTCAGCAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAA

         70        80        90       100       110       120
 GGGGTTATGCTAGTTATTGCTCAGCGGTGGCAGCAGCCAACTCAGCTTCCTTTCGGGCTT

        130       140       150       160       170       180
 TGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGCTTACTAGCAATGATGG
                                             <*  *  C  H  H      HIS
                                            <___ANTI-CD7 ____

        190       200       210       220       230       240
 TGATGATGGTGATCGGCCCCCGAGGCCGAGGAAACGGTGACCGTGGTCCCTGCGCCCCAG
<H  H  H  H  D  A  G  S  A  S  S  V  T  V  T  T  G  A  G  W
<_______________________ANTI-CD7 SCFV________________________

        250       260       270       280       290       300
 ACATCGAGGTACCCCCGTACCTCGTCTCTTGCACAGTAATACATGGCCGTGTCCTCAGAC
<V  D  L  Y  G  R  V  E  D  R  A  C  Y  Y  M  A  T  D  E  S
<_______________________ANTI-CD7 SCFV________________________

        310       320       330       340       350       360
 CTCAGACTGCTCATTTGCAGATACAGGATGTTCCTGGCATTATCTCTGGAGATGGTGAAT
<R  L  S  S  M  Q  L  Y  L  I  N  R  A  N  D  R  S  I  T  F
<_______________________ANTI-CD7 SCFV________________________

        370       380       390       400       410       420
 CGGCCCTTCACACTGTCTGGATAGTAGGTGAAACCACCACTACTAATGGATGCGACCCAC
<R  G  K  V  S  D  P  Y  Y  T  F  G  G  S  S  I  S  A  V  W
<_______________________ANTI-CD7 SCFV________________________

        430       440       450       460       470       480
 TCCAGCCTCTTCTCTGGAGTCTGGCGAACCCAAGACATGGCATAGCTACTGAAAGTGAGT
<E  L  R  K  E  P  T  Q  R  V  W  S  M  A  Y  S  S  F  T  L
<_______________________ANTI-CD7 SCFV________________________

        490       500       510       520       530       540
 CCAGAGGCTGCACAGGAGAGTTTCAGGGACCCCCCAGGCTTCACTAAGCCTCCCCCAGAC
<G  S  A  A  C  S  L  K  L  S  G  G  P  K  V  L  G  G  G  S
<_______________________ANTI-CD7 SCFV________________________

        550       560       570       580       590       600
 TCCACCAGTTGCACCTCGGATCCACCACCACCGGAGCCGCCGCCGCCAGAACCACCACCA
<E  V  L  Q  V  E  S  G  G  G  G  S  G  G  G  G  S  G  G  G
<_______________________ANTI-CD7 SCFV________________________

        610       620       630       640       650       660
 CCAGAACCACCACCACCACGTTTTATTTCCAGCTTGGTCCCCCCTCCGAACGTGTACGGA
<G  S  G  G  G  G  R  K  I  E  L  K  T  G  G  G  F  T  Y  P
<_______________________ANTI-CD7 SCFV________________________

        670       680       690       700       710       720
 AGCTTGCTATACTGCTGACAATAATAAGTGGCAATATCTTCAGGTTCCAGGTTGCTGATG
<L  K  S  Y  Q  Q  C  Y  Y  T  A  I  D  E  P  E  L  N  S  I
```

```
<_______________________ANTI-CD7 SCFV________________________

        730       740       750       760       770       780
 GTGAGAGAATAATCTGTCCCAGACCCACTGCCACTGAACCTTGATGGGACTCCTGAGTGT
<T  L  S  Y  D  T  G  S  G  S  G  S  F  R  S  P  V  G  S  H
<_______________________ANTI-CD7 SCFV________________________

        790       800       810       820       830       840
 AAACTTGATGTGTAATAGATCAGGAGTTTAACAGTTCCATCTGGTTTCTGCTGATACCAG
<L  S  S  T  Y  Y  I  L  L  K  V  T  G  D  P  K  Q  Q  Y  W
<_______________________ANTI-CD7 SCFV________________________

        850       860       870       880       890       900
 TTTAAATAATTGCTAATGCCCTGACTTGCACTGCAACTGATGGTGACTCTGTCTCCCAGA
<N  L  Y  N  S  I  G  Q  S  A  S  C  S  I  T  V  R  D  G  L
<_______________________ANTI-CD7 SCFV________________________

        910       920       930       940       950       960
 GAGGCAGACAGGGAGGATGTAGTCTGTGTCATCTGGATATCTTTGTAGGCCGCCATGGCC
<S  A  S  L  S  S  T  T  Q  T  M  Q  I  D  K  Y  A  A  M  A
<_______________________ANTI-CD7 SCFV________________________

        970       980       990      1000      1010      1020
 GGCTGGGCCGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAGGTATTTCATATGT
<P  Q  A  A  L  L  L  L  G  A  A  A  T  P  L  L  Y  K  M
<_____________________ANTI-CD7 SCFV______________________

       1030      1040      1050      1060      1070      1080
 ATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACA

       1090      1100      1110      1120      1130      1140
 ATTCCCCTATAGTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACGCCGG

       1150      1160      1170      1180      1190      1200
 ACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGA

       1210      1220      1230      1240      1250      1260
 CATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGT

       1270      1280      1290      1300      1310      1320
 GGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCATGCACC

       1330      1340      1350      1360      1370      1380
 ATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTAATGCA

       1390      1400      1410      1420      1430      1440
 GGAGTCGCATAAGGGAGAGCGTCGAGATCCCGGACACCATCGAATGGCGCAAAACCTTTC

       1450      1460      1470      1480      1490      1500
 GCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAG

       1510      1520      1530      1540      1550      1560
 TAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGG

       1570      1580      1590      1600      1610      1620
 TGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGG
```

```
      1630      1640      1650      1660      1670      1680
AGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGA

      1690      1700      1710      1720      1730      1740
TTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTA

      1750      1760      1770      1780      1790      1800
AATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCG

      1810      1820      1830      1840      1850      1860
TCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCA

      1870      1880      1890      1900      1910      1920
TTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTC

      1930      1940      1950      1960      1970      1980
CGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATG

      1990      2000      2010      2020      2030      2040
AAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGC

      2050      2060      2070      2080      2090      2100
TGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAAT

      2110      2120      2130      2140      2150      2160
ATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGT

      2170      2180      2190      2200      2210      2220
CCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGG

      2230      2240      2250      2260      2270      2280
TTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCG

      2290      2300      2310      2320      2330      2340
TTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCC

      2350      2360      2370      2380      2390      2400
CGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCT

      2410      2420      2430      2440      2450      2460
TGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGG

      2470      2480      2490      2500      2510      2520
TGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCG

      2530      2540      2550      2560      2570      2580
ATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAAC

      2590      2600      2610      2620      2630      2640
GCAATTAATGTAAGTTAGCTCACTCATTAGGCACCGGGATCTCGACCGATGCCCTTGAGA

      2650      2660      2670      2680      2690      2700
GCCTTCAACCCAGTCAGCTCCTTCCGGTGGGCGCGGGGCATGACTATCGTCGCCGCACTT

      2710      2720      2730      2740      2750      2760
ATGACTGTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATT
```

```
      2770      2780      2790      2800      2810      2820
TTCGGCGAGGACCGCTTTCGCTGGAGCGCGACGATGATCGGCCTGTCGCTTGCGGTATTC

      2830      2840      2850      2860      2870      2880
GGAATCTTGCACGCCCTCGCTCAAGCCTTCGTCACTGGTCCCGCCACCAAACGTTTCGGC

      2890      2900      2910      2920      2930      2940
GAGAAGCAGGCCATTATCGCCGGCATGGCGGCCCCACGGGTGCGCATGATCGTGCTCCTG

      2950      2960      2970      2980      2990      3000
TCGTTGAGGACCCGGCTAGGCTGGCGGGGTTGCCTTACTGGTTAGCAGAATGAATCACCG

      3010      3020      3030      3040      3050      3060
ATACGCGAGCGAACGTGAAGCGACTGCTGCTGCAAAACGTCTGCGACCTGAGCAACAACA

      3070      3080      3090      3100      3110      3120
TGAATGGTCTTCGGTTTCCGTGTTTCGTAAAGTCTGGAAACGCGGAAGTCAGCGCCCTGC

      3130      3140      3150      3160      3170      3180
ACCATTATGTTCCGGATCTGCATCGCAGGATGCTGCTGGCTACCCTGTGGAACACCTACA

      3190      3200      3210      3220      3230      3240
TCTGTATTAACGAAGCGCTGGCATTGACCCTGAGTGATTTTTCTCTGGTCCCGCCGCATC

      3250      3260      3270      3280      3290      3300
CATACCGCCAGTTGTTTACCCTCACAACGTTCCAGTAACCGGGCATGTTCATCATCAGTA

      3310      3320      3330      3340      3350      3360
ACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGGTATCATTACCCCCATGAACAGAAAT

      3370      3380      3390      3400      3410      3420
CCCCCTTACACGGAGGCATCAGTGACCAAACAGGAAAAAACCGCCCTTAACATGGCCCGC

      3430      3440      3450      3460      3470      3480
TTTATCAGAAGCCAGACATTAACGCTTCTGGAGAAACTCAACGAGCTGGACGCGGATGAA

      3490      3500      3510      3520      3530      3540
CAGGCAGACATCTGTGAATCGCTTCACGACCACGCTGATGAGCTTTACCGCAGCTGCCTC

      3550      3560      3570      3580      3590      3600
GCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACA

      3610      3620      3630      3640      3650      3660
GCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTT

      3670      3680      3690      3700      3710      3720
GGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGC

      3730      3740      3750      3760      3770      3780
TTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAAT

      3790      3800      3810      3820      3830      3840
ACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTCGCTCAC

      3850      3860      3870      3880      3890      3900
TGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGT
```

```
      3910      3920      3930      3940      3950      3960
AATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCA

      3970      3980      3990      4000      4010      4020
GCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC

      4030      4040      4050      4060      4070      4080
CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACT

      4090      4100      4110      4120      4130      4140
ATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCT

      4150      4160      4170      4180      4190      4200
GCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAG

      4210      4220      4230      4240      4250      4260
CTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCA

      4270      4280      4290      4300      4310      4320
CGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAA

      4330      4340      4350      4360      4370      4380
CCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC

      4390      4400      4410      4420      4430      4440
GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAG

      4450      4460      4470      4480      4490      4500
AAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGG

      4510      4520      4530      4540      4550      4560
TAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCA

      4570      4580      4590      4600      4610      4620
GCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTC

      4630      4640      4650      4660      4670      4680
TGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAACAATAAAACTGT

      4690      4700      4710      4720      4730      4740
CTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTT

      4750      4760      4770      4780      4790      4800
GCTCTAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTC

      4810      4820      4830      4840      4850      4860
GCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGC

      4870      4880      4890      4900      4910      4920
CAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGG

      4930      4940      4950      4960      4970      4980
TCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTA

      4990      5000      5010      5020      5030      5040
CTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTAT
```

```
       5050      5060      5070      5080      5090      5100
TAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCC

       5110      5120      5130      5140      5150      5160
GGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCG

       5170      5180      5190      5200      5210      5220
CTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGC

       5230      5240      5250      5260      5270      5280
GTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCAC

       5290      5300      5310      5320      5330      5340
CGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGA

       5350      5360      5370      5380      5390      5400
AATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTG

       5410      5420      5430      5440      5450      5460
CCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAA

       5470      5480      5490      5500      5510      5520
AATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGT

       5530      5540      5550      5560      5570      5580
TTTTCTAAGAATTAATTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAA

       5590      5600      5610      5620      5630      5640
ATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAAATTGTAAACGTTAATATT

       5650      5660      5670      5680      5690      5700
TTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAA

       5710      5720      5730      5740      5750      5760
ATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCA

       5770      5780      5790      5800      5810      5820
GTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACC

       5830      5840      5850      5860      5870      5880
GTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCG

       5890      5900      5910      5920      5930      5940
AGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGG

       5950      5960      5970      5980      5990      6000
GGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGG

       6010      6020      6030      6040      6050      6060
GCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCG

       6070      6080
CCGCTACAGGGCGCGTCCCATTCGCCA
```

C. Sequence of VKExpress anti-CD7 VL plasmid

```
        10        20        30        40        50        60
CATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGC
 M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A>
 _______________________ANTI-CD7 VL_________________________>

        70        80        90       100       110       120
CATGGCGGCCTACAAAGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCT
 M  A  A  Y  K  D  I  Q  M  T  Q  T  T  S  S  L  S  A  S  L>
________________________ANTI-CD7 VL_________________________>

       130       140       150       160       170       180
GGGAGACAGAGTCACCATCAGTTGCAGTGCAAGTCAGGGCATTAGCAATTATTTAAACTG
 G  D  R  V  T  I  S  C  S  A  S  Q  G  I  S  N  Y  L  N  W>
________________________ANTI-CD7 VL_________________________>

       190       200       210       220       230       240
GTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTATTACACATCAAGTTTACA
 Y  Q  Q  K  P  D  G  T  V  K  L  L  I  Y  Y  T  S  S  L  H>
________________________ANTI-CD7 VL_________________________>

       250       260       270       280       290       300
CTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGGACAGATTATTCTCTCACCAT
 S  G  V  P  S  R  F  S  G  S  G  S  G  T  D  Y  S  L  T  I>
________________________ANTI-CD7 VL_________________________>

       310       320       330       340       350       360
CAGCAACCTGGAACCTGAAGATATTGCCACTTATTATTGTCAGCAGTATAGCAAGCTTCC
 S  N  L  E  P  E  D  I  A  T  Y  Y  C  Q  Q  Y  S  K  L  P>
________________________ANTI-CD7 VL_________________________>

       370       380       390       400       410       420
GTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAACGTCGTGAGTAGAATAGATCTAA
 Y  T  F  G  G  G  T  K  L  E  I  K  R>
______________ANTI-CD7 VL_______________>

       430       440       450       460       470       480
CTTAATTAAGGAATAGGGGAAGCTAGGAAGAAACTCAAAACATCAAGATTTTAAATACGC

       490       500       510       520       530       540
TTCTTGGTCTCCTTGCTATAATTATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAA

       550       560       570       580       590       600
CATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCAGAACTTTGTTACTTAAACAC

       610       620       630       640       650       660
CATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGC
                           T  V  A  A  P  S  V  F  I  F  P>

       670       680       690       700       710       720
CATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT
P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  N  N  F>

       730       740       750       760       770       780
ATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCC
```

```
Y  P  R  E  A  K  V  Q  W  K  V  D  N  A  L  Q  S  G  N  S>

       790       800       810       820       830       840
AGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S  S  T  L>

       850       860       870       880       890       900
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGG
T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T  H  Q>

       910       920       930       940       950       960
GCCTGAGTTCACCGGTGACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGACTAGTGCC
G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  *>

       970       980       990      1000      1010      1020
CCCACCTGCTCCTCAGTTCCAGCCTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTT

      1030      1040      1050      1060      1070      1080
CCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCTCATCTTTCACCTCACCCCCCTCCT

      1090      1100      1110      1120      1130      1140
CCTCCTTGGCTTTAATTATGCTAATGTTGGAGGAGAATGAATAAATAAAGTGAATCTTTG

      1150      1160      1170      1180      1190      1200
CACCTGTGGTTTCTCTCTTTCCTCATTTAATAATTATTATCTGTTGTTTTACCAACTACT

      1210      1220      1230      1240      1250      1260
CAATTTCTCTTATAAGGGACTAAATATGTAGTCATCCTAAGGCGCATAACCATTTATAAA

      1270      1280      1290      1300      1310      1320
AATCATCCTTCATTCTATTTACCCTATCATCCTCTGCAAGACAGTCCTCCCTCAAACCCA

      1330      1340      1350      1360      1370      1380
CAAGCCTTCTGTCCTCACAGTCCCCCTGGGCCGTGGTAGGAGAGACTTGCTTCCTTGTTT

      1390      1400      1410      1420      1430      1440
TCCCCTCCTCAGCAAGCCCTCATAGTCCTTTTTAAGGGTGACAGGTCTTACGGTCATATA

      1450      1460      1470      1480      1490      1500
TCCTTTGATTCAATAAGCTTGGTACCAGCCCGGGCACGCGTCAGTCTAGAAGCTCGCTGA

      1510      1520      1530      1540      1550      1560
TCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCT

      1570      1580      1590      1600      1610      1620
TCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCA

      1630      1640      1650      1660      1670      1680
TCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAG

      1690      1700      1710      1720      1730      1740
GGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGGCCCGGGCTCTATGGCTTCT

      1750      1760      1770      1780      1790      1800
GAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCA

      1810      1820      1830      1840      1850      1860
```

```
TTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTA

      1870      1880      1890      1900      1910      1920
GCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGT

      1930      1940      1950      1960      1970      1980
CAAGCTCTAAATCGGGGCATCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGAC

      1990      2000      2010      2020      2030      2040
CCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTT

      2050      2060      2070      2080      2090      2100
TTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGA

      2110      2120      2130      2140      2150      2160
ACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGGGGATTTCG

      2170      2180      2190      2200      2210      2220
GCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGA

      2230      2240      2250      2260      2270      2280
ATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGGCAGGCAGAAGTATGCAA

      2290      2300      2310      2320      2330      2340
AGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGC

      2350      2360      2370      2380      2390      2400
AGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCG

      2410      2420      2430      2440      2450      2460
CCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCTAGGCTGACTAATT

      2470      2480      2490      2500      2510      2520
TTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGA

      2530      2540      2550      2560      2570      2580
GGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTCCCGGGCCACCATGAGCGAAAA

      2590      2600      2610      2620      2630      2640
ATACATCGTCACCTGGGACATGTTGCAGATCCATGCACGTAAACTCGCAAGCCGACTGAT

      2650      2660      2670      2680      2690      2700
GCCTTCTGAACAATGGAAAGGCATTATTGCCGTAAGCCGTGGCGGTCTGGTACCGGGTGC

      2710      2720      2730      2740      2750      2760
GTTACTGGCGCGTGAACTGGGTATTCGTCATGTCGATACCGTTTGTATTTCCAGCTACGA

      2770      2780      2790      2800      2810      2820
TCACGACAACCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGCGATGGCGAAGGCTT

      2830      2840      2850      2860      2870      2880
CATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATTCGTGAAATGTA

      2890      2900      2910      2920      2930      2940
TCCAAAAGCGCACTTTGTCACCATCTTCGCAAAACCGGCTGGTCGTCCGCTGGTTGATGA

      2950      2960      2970      2980      2990      3000
```

```
CTATGTTGTTGATATCCCGCAAGATACCTGGATTGAACAGCCGTGGGATATGGGCGTCGT

      3010      3020      3030      3040      3050      3060
ATTCGTCCCGCCAATCTCCGGTCGCTAATCTTTTCGAAATGACCGACCAAGCGACGCCCA

      3070      3080      3090      3100      3110      3120
ACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAA

      3130      3140      3150      3160      3170      3180
TCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCT

      3190      3200      3210      3220      3230      3240
TCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCA

      3250      3260      3270      3280      3290      3300
CAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCA

      3310      3320      3330      3340      3350      3360
TCAATGTATCTTATCATGTCTGTATACCGGATCTTTCCGCTTCCTCGCTCACTGACTCGC

      3370      3380      3390      3400      3410      3420
TGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGT

      3430      3440      3450      3460      3470      3480
TATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGG

      3490      3500      3510      3520      3530      3540
CCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACG

      3550      3560      3570      3580      3590      3600
AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGAT

      3610      3620      3630      3640      3650      3660
ACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTA

      3670      3680      3690      3700      3710      3720
CCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCT

      3730      3740      3750      3760      3770      3780
GTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCC

      3790      3800      3810      3820      3830      3840
CCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAA

      3850      3860      3870      3880      3890      3900
GACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATG

      3910      3920      3930      3940      3950      3960
TAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAG

      3970      3980      3990      4000      4010      4020
TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT

      4030      4040      4050      4060      4070      4080
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTA

      4090      4100      4110      4120      4130      4140
```

```
CGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTC

      4150      4160      4170      4180      4190      4200
AGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCA

      4210      4220      4230      4240      4250      4260
CCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA

      4270      4280      4290      4300      4310      4320
CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTAT

      4330      4340      4350      4360      4370      4380
TTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCT

      4390      4400      4410      4420      4430      4440
TACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT

      4450      4460      4470      4480      4490      4500
TATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTAT

      4510      4520      4530      4540      4550      4560
CCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTA

      4570      4580      4590      4600      4610      4620
ATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTG

      4630      4640      4650      4660      4670      4680
GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGT

      4690      4700      4710      4720      4730      4740
TGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCG

      4750      4760      4770      4780      4790      4800
CAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCG

      4810      4820      4830      4840      4850      4860
TAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGC

      4870      4880      4890      4900      4910      4920
GGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAA

      4930      4940      4950      4960      4970      4980
CTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTAC

      4990      5000      5010      5020      5030      5040
CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTT

      5050      5060      5070      5080      5090      5100
TTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGG

      5110      5120      5130      5140      5150      5160
GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAA

      5170      5180      5190      5200      5210      5220
GCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATA

      5230      5240      5250      5260      5270      5280
```

```
AACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCAGATCGACGG

      5290      5300      5310      5320      5330      5340
ATCGGGAGATCGGTACCTAGAATTCGCCCGGGCAGCTTCGTGAGGCTCCGGTGCCCGTCA

      5350      5360      5370      5380      5390      5400
GTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTG

      5410      5420      5430      5440      5450      5460
AACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCT

      5470      5480      5490      5500      5510      5520
CCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGT

      5530      5540      5550      5560      5570      5580
TCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGG

      5590      5600      5610      5620      5630      5640
GCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTCCA

      5650      5660      5670      5680      5690      5700
GTACGTGATTCTTGATCCCGAGCTGGAGCCAGGGGCGGGCCTTGCGCTTTAGGAGCCCCT

      5710      5720      5730      5740      5750      5760
TCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGG

      5770      5780      5790      5800      5810      5820
TGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGA

      5830      5840      5850      5860      5870      5880
TGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAGGATCTG

      5890      5900      5910      5920      5930      5940
CACACTGGTATTTCGGTTTTTGGGCCCGCGGCCGGCGACGGGGCCCGTGCGTCCCAGCGC

      5950      5960      5970      5980      5990      6000
ACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCT

      6010      6020      6030      6040      6050      6060
CAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGG

      6070      6080      6090      6100      6110      6120
GCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGC

      6130      6140      6150      6160      6170      6180
CCTGCTCCAGGGGGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCA

      6190      6200      6210      6220      6230      6240
CCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAG

      6250      6260      6270      6280      6290      6300
TACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTGGAGCTTTTGGAGTACGTCGTCTTTA

      6310      6320      6330      6340      6350      6360
GGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAA

      6370      6380      6390      6400      6410      6420
```

```
GTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGGCCTTTTTGAGTTTGGA

      6430      6440      6450      6460      6470      6480
TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTG

      6490      6500      6510      6520      6530      6540
TCGTGAACACGTACGTACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGC

      6550      6560      6570      6580      6590      6600
TACAGGTAAGGGGTTAACAGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATG

      6610      6620      6630      6640
ACATCCACTTTGCCTTTCTCTCCACAGGCGTGCACTCCGATATCGAGCT
```

D. Sequence of VHExpress anti-CD7 VH plasmid

```
        10        20        30        40        50        60
GAGGTGCAACTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGGGGGTCCCTGAAACTC
 E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L  K  L>
________________________ANTI-CD7 VH_________________________>

        70        80        90       100       110       120
TCCTGTGCAGCCTCTGGACTCACTTTCAGTAGCTATGCCATGTCTTGGGTTCGCCAGACT
 S  C  A  A  S  G  L  T  F  S  S  Y  A  M  S  W  V  R  Q  T>
________________________ANTI-CD7 VH_________________________>

       130       140       150       160       170       180
CCAGAGAAGAGGCTGGAGTGGGTCGCATCCATTAGTAGTGGTGGTTTCACCTACTATCCA
 P  E  K  R  L  E  W  V  A  S  I  S  S  G  G  F  T  Y  Y  P>
________________________ANTI-CD7 VH_________________________>

       190       200       210       220       230       240
GACAGTGTGAAGGGCCGATTCACCATCTCCAGAGATAATGCCAGGAACATCCTGTATCTG
 D  S  V  K  G  R  F  T  I  S  R  D  N  A  R  N  I  L  Y  L>
________________________ANTI-CD7 VH_________________________>

       250       260       270       280       290       300
CAAATGAGCAGTCTGAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGAGACGAGGTA
 Q  M  S  S  L  R  S  E  D  T  A  M  Y  Y  C  A  R  D  E  V>
________________________ANTI-CD7 VH_________________________>

       310       320       330       340       350       360
CGGGGGTACCTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTTTCCTCGGCCTCGGGG
 R  G  Y  L  D  V  W  G  A  G  T  T  V  T  V  S  S  A  S  G>
________________________ANTI-CD7 VH_________________________>

       370       380       390       400       410       420
GCCGATGGTGAGTCCTGTCGACGGATCCACCCAATGCCCATGAGCCCAGACACTGGACGC
 A  D>
_______>

       430       440       450       460       470       480
TGAACCTCGCGGACAGTTAAGAACCCAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCC
```

```
       490       500       510       520       530       540
ACACCGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTC
                                  A  S  T  K  G  P  S  V  F>

       550       560       570       580       590       600
CCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC
 P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V>

       610       620       630       640       650       660
AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGC
 K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G>

       670       680       690       700       710       720
GTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTAGTG
 V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V>

       730       740       750       760       770       780
ACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCC
 T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P>

       790       800       810       820       830       840
AGCAACACCAAGGTGGACAAGAAAGTTGGTGAGAGGCCAGCACAGGGAGGGAGGGTGTCT
 S  N  T  K  V  D  K  K  V>

       850       860       870       880       890       900
GCTGGAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCA

       910       920       930       940       950       960
GGGCAGCAAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCAT

       970       980       990      1000      1010      1020
GCTCAGGGAGAGGGTCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACAGGCTAGGTGC

      1030      1040      1050      1060      1070      1080
CCCTAACCCAGGCCCTGCACACAAAGGGGCTGGTGCTGGGCTCAGACCTGCCAAGAGCCA

      1090      1100      1110      1120      1130      1140
TATCCGGGAGGACCCTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCC

      1150      1160      1170      1180      1190      1200
TCAGCTCGGACACCTTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTCCAGA
                                                           E>

      1210      1220      1230      1240      1250      1260
GCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGGTAAGCCAGCCCAGGC
  P  K  S  C  D  K  T  H  T  C  P  P  C  P>

      1270      1280      1290      1300      1310      1320
CTCGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGGGTAGCCTGCATCCAGGGACAGGC

      1330      1340      1350      1360      1370      1380
CCCAGCCGGGTGCTGACACGTCCACCTCCATCTCTTCCTCAGCACCTGAACTCCTGGGGG
                                          A  P  E  L  L  G>

      1390      1400      1410      1420      1430      1440
GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T>
```

```
      1450      1460      1470      1480      1490      1500
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACT
P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N>

      1510      1520      1530      1540      1550      1560
GGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACA
W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y>

      1570      1580      1590      1600      1610      1620
ACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G>

      1630      1640      1650      1660      1670      1680
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCT
K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I>

      1690      1700      1710      1720      1730      1740
CCAAAGCCAAAGGTGGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGC
S  K  A  K>

      1750      1760      1770      1780      1790      1800
CCACCCTCTGCCCTGAGAGTGACCGCTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGA
                                                 G  Q  P  R>

      1810      1820      1830      1840      1850      1860
GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGC
 E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S>

      1870      1880      1890      1900      1910      1920
CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
 L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N>

      1930      1940      1950      1960      1970      1980
GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC
 G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F>

      1990      2000      2010      2020      2030      2040
TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA
 F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S>

      2050      2060      2070      2080      2090      2100
TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCTTAAGT
 C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S>

      2110      2120      2130      2140      2150      2160
CCGGGAAAATAATCTAGAAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCC
 P  G  K>

      2170      2180      2190      2200      2210      2220
ATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGT

      2230      2240      2250      2260      2270      2280
CCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCT

      2290      2300      2310      2320      2330      2340
GGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGC
```

```
      2350      2360      2370      2380      2390      2400
TGGGGATGGCCCGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGG

      2410      2420      2430      2440      2450      2460
GTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAG

      2470      2480      2490      2500      2510      2520
CGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTT

      2530      2540      2550      2560      2570      2580
TCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGCATCCCTTTAGGGTT

      2590      2600      2610      2620      2630      2640
CCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACG

      2650      2660      2670      2680      2690      2700
TAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTT

      2710      2720      2730      2740      2750      2760
TAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTT

      2770      2780      2790      2800      2810      2820
TGATTTATAAGGGATTTTGGGGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACA

      2830      2840      2850      2860      2870      2880
AAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCA

      2890      2900      2910      2920      2930      2940
GGCTCCCCAGGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTG

      2950      2960      2970      2980      2990      3000
TGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTC

      3010      3020      3030      3040      3050      3060
AGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGC

      3070      3080      3090      3100      3110      3120
CCATTCTCCGCCCCTAGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTC

      3130      3140      3150      3160      3170      3180
TGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAA

      3190      3200      3210      3220      3230      3240
AAAGCTCCCGGGAGGTCCACAATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCC

      3250      3260      3270      3280      3290      3300
GCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT

      3310      3320      3330      3340      3350      3360
GCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTG

      3370      3380      3390      3400      3410      3420
TCCGGTGCCCTGAATGAACTCCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACG

      3430      3440      3450      3460      3470      3480
GGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTA
```

```
      3490      3500      3510      3520      3530      3540
TTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTA

      3550      3560      3570      3580      3590      3600
TCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTC

      3610      3620      3630      3640      3650      3660
GACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTC

      3670      3680      3690      3700      3710      3720
GATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGG

      3730      3740      3750      3760      3770      3780
CTCAAGGCGCGTATGCCCGACGGCGAGGATCTCGTCGTGACTCATGGCGATGCCTGCTTG

      3790      3800      3810      3820      3830      3840
CCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGT

      3850      3860      3870      3880      3890      3900
GTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGC

      3910      3920      3930      3940      3950      3960
GGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGC

      3970      3980      3990      4000      4010      4020
ATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGA

      4030      4040      4050      4060      4070      4080
CCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATG

      4090      4100      4110      4120      4130      4140
AAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGG

      4150      4160      4170      4180      4190      4200
ATCTCATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACA

      4210      4220      4230      4240      4250      4260
AATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTT

      4270      4280      4290      4300      4310      4320
GTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGGATCTTTCCGCTT

      4330      4340      4350      4360      4370      4380
CCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACT

      4390      4400      4410      4420      4430      4440
CAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAG

      4450      4460      4470      4480      4490      4500
CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATA

      4510      4520      4530      4540      4550      4560
GGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC

      4570      4580      4590      4600      4610      4620
CGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTG
```

```
      4630      4640      4650      4660      4670      4680
TTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGC

      4690      4700      4710      4720      4730      4740
TTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGG

      4750      4760      4770      4780      4790      4800
GCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTC

      4810      4820      4830      4840      4850      4860
TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA

      4870      4880      4890      4900      4910      4920
TTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACG

      4930      4940      4950      4960      4970      4980
GCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA

      4990      5000      5010      5020      5030      5040
AAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTG

      5050      5060      5070      5080      5090      5100
TTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT

      5110      5120      5130      5140      5150      5160
CTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGAT

      5170      5180      5190      5200      5210      5220
TATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCT

      5230      5240      5250      5260      5270      5280
AAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTA

      5290      5300      5310      5320      5330      5340
TCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAA

      5350      5360      5370      5380      5390      5400
CTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCAC

      5410      5420      5430      5440      5450      5460
GCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAA

      5470      5480      5490      5500      5510      5520
GTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAG

      5530      5540      5550      5560      5570      5580
TAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGG

      5590      5600      5610      5620      5630      5640
TGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAG

      5650      5660      5670      5680      5690      5700
TTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTG

      5710      5720      5730      5740      5750      5760
TCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTC
```

```
      5770      5780      5790      5800      5810      5820
TTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCAT

      5830      5840      5850      5860      5870      5880
TCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATA

      5890      5900      5910      5920      5930      5940
CCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAA

      5950      5960      5970      5980      5990      6000
AACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCA

      6010      6020      6030      6040      6050      6060
ACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGC

      6070      6080      6090      6100      6110      6120
AAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCC

      6130      6140      6150      6160      6170      6180
TTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTG

      6190      6200      6210      6220      6230      6240
AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC

      6250      6260      6270      6280      6290      6300
CTGACGTCAGATCGACGGATCGGGAGATCAGGTACCGAATTCAAGCTTCGTGAGGCTCCG

      6310      6320      6330      6340      6350      6360
GTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGG

      6370      6380      6390      6400      6410      6420
TCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCG

      6430      6440      6450      6460      6470      6480
TGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCG

      6490      6500      6510      6520      6530      6540
CCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTG

      6550      6560      6570      6580      6590      6600
GTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCA

      6610      6620      6630      6640      6650      6660
CCTGGCTCCAGTACGTGATTCTTGATCCCGAGCTGGAGCCAGGGGCGGGCCTTGCGCTTT

      6670      6680      6690      6700      6710      6720
AGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGT

      6730      6740      6750      6760      6770      6780
GCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTA

      6790      6800      6810      6820      6830      6840
AAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGG

      6850      6860      6870      6880      6890      6900
CCAGGATCTGCACACTGGTATTTCGGTTTTTGGGCCCGCGGCCGGCGACGGGGCCCGTGC
```

```
      6910      6920      6930      6940      6950      6960
GTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACG

      6970      6980      6990      7000      7010      7020
GGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC

      7030      7040      7050      7060      7070      7080
CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCC

      7090      7100      7110      7120      7130      7140
GCTTCCCGGCCCTGCTCCAGGGGGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGC

      7150      7160      7170      7180      7190      7200
GGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGA

      7210      7220      7230      7240      7250      7260
CTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTGGAGCTTTTGGAGTAC

      7270      7280      7290      7300      7310      7320
GTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGT

      7330      7340      7350      7360      7370      7380
GGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGGCCTTTT

      7390      7400      7410      7420      7430      7440
TGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCC

      7450      7460      7470      7480      7490      7500
ATTTCAGGTGTCGTGAACACGTGGCCACCATGGGATGGAGCTGTATCATCCTCTTCTTGG

      7510      7520      7530      7540      7550      7560
TAGCAACAGCTACAGGTAAGGGGTTAACAGTAGCAGGCTTGAGGTCTGGACATATATATG

      7570      7580      7590      7600
GGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGCGCGCACTCC
```

Supplementary Table 1. The number of cell surface CD7 molecules on cell lines

Cell line	Number of cell surface CD7 molecules
A549	99
A549-CD7	144,000
MOLT4	10,900
Jurkat	7960
KOPT-KI	21,400

The number of cell surface CD7 molecules was estimated using BD Quantibrite Beads

Supplementary Table 2: Reduction and conjugation conditions used to generate CD7-PEG4-Val-Cit-PAB-MMAE

Reduction Step (2 hours at 25 ˚C)		
Component	*Volume*	*Final Concentration*
CD7 (96.4 µM, 14.48 mg/mL) in PBS + 4 mM EDTA, pH 7.40	1.15 mL	73.9 µM
TCEP (40 mM) in in PBS + 4 mM EDTA, pH 7.40	8.3 µL	221.3 µM (3 molar equivalents)
PBS + 4 mM EDTA, pH 7.40	342 µL	N/A
Total volume	**1.5 mL**	
Conjugation Step (21 hours at 25 ˚C)		
Component	*Volume*	*Final Concentration*
Partially reduced CD7 (73.9 µM) in PBS + 4 mM EDTA, pH 7.40	1.5 mL	66.5 µM
Dimethylacetamide (DMA)	67 µL	10% (v/v)
PermaLink-PEG4-Val-Cit-PAB-MMAE (25 mM) in DMA	100 µL	1.5 mM (22.5 molar equivalents)
Total volume	**1.667 mL**	

Supplementary Table 3: PLRP-HPLC method information

<table>
<tr><th colspan="3">PLRP-HPLC method information</th></tr>
<tr><td>Component</td><td colspan="2">Parameter</td></tr>
<tr><td>Sample preparation</td><td colspan="2">ADC was incubated for 15 min at 37 ˚C in the presence of 2 mM DTT immediately prior to analysis</td></tr>
<tr><td>Column</td><td colspan="2">PLRP-S (Agilent, 1000 Å, 5 µm, 50 x 2.1 mm)</td></tr>
<tr><td>Column temperature</td><td colspan="2">80 °C</td></tr>
<tr><td>Flow rate</td><td colspan="2">0.75 mL/min</td></tr>
<tr><td>Injection Volume</td><td colspan="2">20 µL</td></tr>
<tr><td>Detection</td><td colspan="2">A280 nm and A254 nm</td></tr>
<tr><td>Mobile phase A</td><td colspan="2">0.1% (v/v) TFA in deionised water</td></tr>
<tr><td>Mobile phase B</td><td colspan="2">0.1% (v/v) TFA in Acetonitrile</td></tr>
<tr><td rowspan="8">Gradient</td><td>Time (min)</td><td>Mobile phase B (%)</td></tr>
<tr><td>0</td><td>25</td></tr>
<tr><td>3</td><td>25</td></tr>
<tr><td>28</td><td>50</td></tr>
<tr><td>29</td><td>95</td></tr>
<tr><td>31</td><td>95</td></tr>
<tr><td>33</td><td>25</td></tr>
<tr><td>42</td><td>25</td></tr>
</table>

Supplementary Table 4: Reduction and conjugation conditions used to generate Herceptin-PEG4-Val-Cit-PAB-MMAE

Reduction Step (2 hours at 25 ˚C)		
Component	*Volume*	*Final Concentration*
Herceptin (79 µM, 11.9 mg/mL) in PBS + 4 mM EDTA, pH 7.40	200 µL	44.2 µM
TCEP (21.4 mM) in in PBS + 4 mM EDTA, pH 7.40	2.2 µL	131.9 µM (3 molar equivalents)
PBS + 4 mM EDTA, pH 7.40	154.8 µL	N/A
Total volume	**357 µL**	
Conjugation Step (21 hours at 25 ˚C)		
Component	*Volume*	*Final Concentration*
Partially reduced Herceptin (44.2 µM) in PBS + 4 mM EDTA, pH 7.40	350 µL	39.8 µM
Dimethylacetamide (DMA)	15.7 µL	10% (v/v)
PermaLink-PEG4-Val-Cit-PAB-MMAE (25 mM) in DMA	23.3 µL	1.5 mM (38 molar equivalents)
Total volume	**389 µL**	