

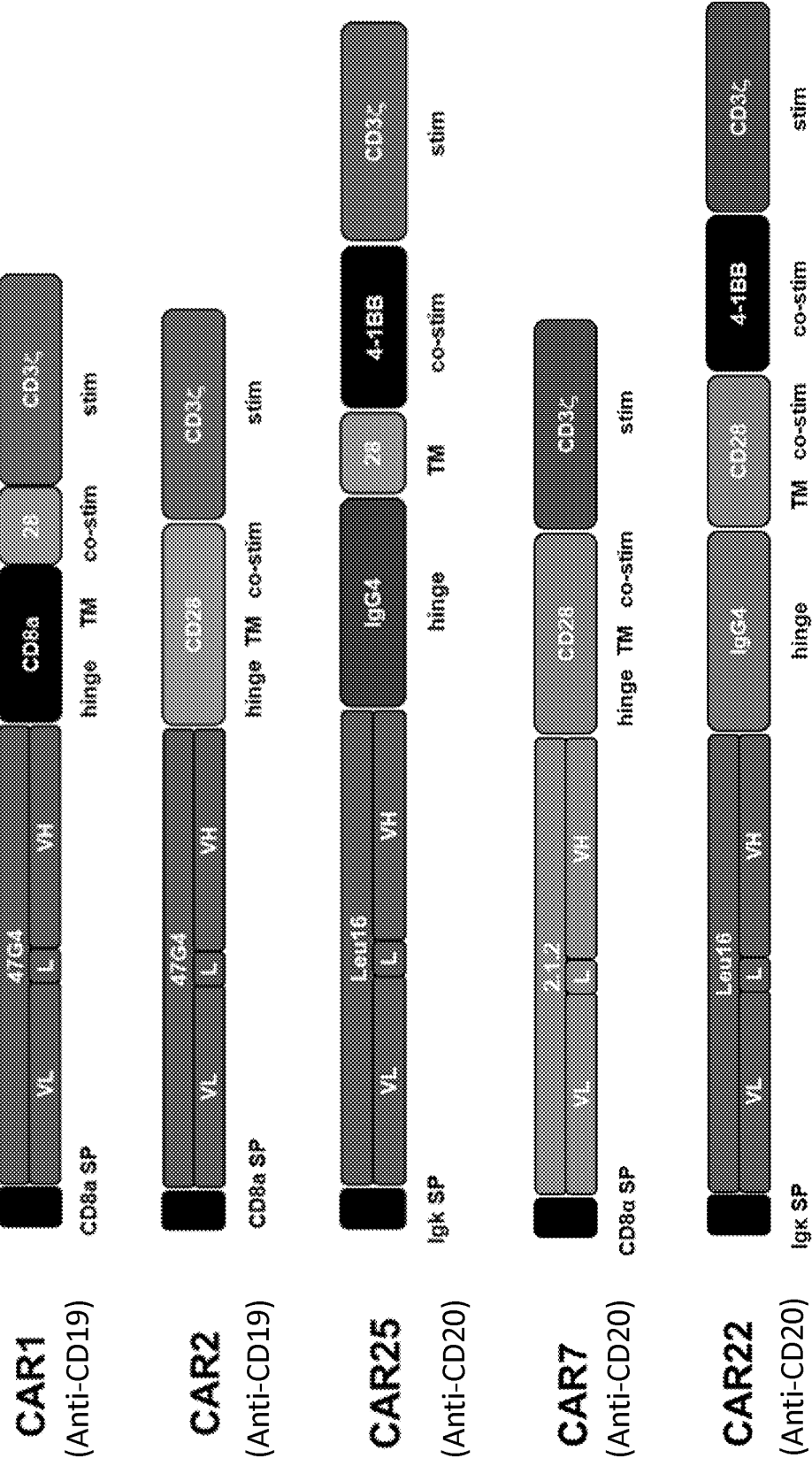


FIG. 1

| SEQ ID NO. | CAR construct | Amino acid sequence (CAR + SP)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Note                                                                                |
|------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| 1          | CAR1          | MALPVTALLPLALLLHAARPEIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRFTFGPGTKVDIKGSTSGSGKPGSGEGSTKGQVQLVQSGAEVKKPGSSVKVCKDSSGTFSSYAISWVRQAPGQGLEWMGGIPIFGTTNYAQQFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREAVAADWLDPWGQGTLVTVSSFPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAFLAGTCGVLLLSLVITLYCNHRNRKRRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                    | Length (whole): 502<br>Length (CAR): 481<br>Diff: 21<br>Source: Genebank QHQ73568.1 |
| 2          | CAR2          | MALPVTALLPLALLLHAARPEIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRFTFGPGTKVDIKGSTSGSGKPGSGEGSTKGQVQLVQSGAEVKKPGSSVKVCKDSSGTFSSYAISWVRQAPGQGLEWMGGIPIFGTTNYAQQFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREAVAADWLDPWGQGTLVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVGGLACYSLLVTVAFIIFWVRSKRRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                      | Length (whole): 485<br>Length (CAR): 464<br>Diff: 21<br>Source: Genebank QHQ73565.1 |
| 3          | CAR25         | METDTLLLWVLLLWVPGSTGDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQQKPGSSPKPWYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWFSNPPTFGGGTKLEIKGSTSGGGSGGGSGGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFVDVWGAGTTVTVSSESKYGPCCPPCPAPEFDGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHLEHNHYTQKSLSLGLKMFVVLVVGGLACYSLLTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR | Length (whole): 677<br>Length (CAR): 657<br>Diff: 20                                |

FIG. 2A

| SEQ ID NO. | CAR construct | Amino acid sequence (CAR + SP)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Note                                                          |
|------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|
| 74         | CAR7          | MALPVTALLLPLALLLHAARPDIVMTQTPHSSPVTLGQPASISCRSS<br>QSLVSRDGNITYLSWLQQRPGQPPRLIYKISNRFSGVPNRFSGSG<br>AGTDFTLKISRVKAEDVGYYCMQATQFPLTFGQGTRLEIKGGGG<br>SGGGGSGGGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWI<br>GWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQGGQVTISADKSIST<br>AYLQWSSLKASDTAMYYCARQGD FWSGYGGMDVWGQGT TVT<br>VSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP LFPGPSKPFWVL<br>VVVGGVLACYSLLVTVAFIIFWVR SKRSRLHSDYMNMTPRRPGP<br>TRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAE<br>AYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                                 | Length<br>(whole):<br>489<br>Length<br>(CAR): 468<br>Diff: 21 |
| 75         | CAR22         | METDTLLLWVLLLWVPGSTGDIVLTQSPAILSASPGEKVTMTCRAS<br>SSVNYMDWYQKKPGSSPKWIYATSNLASGV PARFSGSGSGTSY<br>SLTISRVEAEDAATYYCQQW SFNPPTFGGG TKLEIKGSTSGGGSG<br>GGSGGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNM<br>HWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSST<br>AYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSSL<br>DESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV<br>VDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL<br>TVLHQDWLNGKEYCKVSNKGLPSSIEKTISKAKGQPREPQVYTL P<br>PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP<br>VLDS DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSL<br>SLSLGKMFVVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRGGHSDY<br>MNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSADAPAYQQGQ<br>NQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNE<br>LQDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALH<br>MQALPPR | Length<br>(whole):<br>720<br>Length<br>(CAR): 700<br>Diff: 20 |

FIG. 2A (cont'd)

| SEQ ID NO. | CAR construct | Amino acid sequence (mature CAR)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4          | CAR1          | EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASS<br>RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRFTFGPGTKVDIK<br>GSTSGSGKPGSGEGSTKGQVQLVQSGAEVKKPGSSVKVSCDKSGGTFSSYAIS<br>WVRQAPGQGLEWMGGIIPFGTTNYAQQFQGRVTITADESTSTAYMELSSL<br>RSED TAVYYCAREAVAADWLDPWGQGT LVT VSS FVPVFLPAKPTTTPAPRPP<br>TPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL<br>VITLYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR<br>VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR<br>KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYD<br>ALHMQALPPR                                                                                                                                                                                             |
| 5          | CAR2          | EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASS<br>RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRFTFGPGTKVDIK<br>GSTSGSGKPGSGEGSTKGQVQLVQSGAEVKKPGSSVKVSCDKSGGTFSSYAIS<br>WVRQAPGQGLEWMGGIIPFGTTNYAQQFQGRVTITADESTSTAYMELSSL<br>RSED TAVYYCAREAVAADWLDPWGQGT LVT VSS IEV MYPPPYLDNEKSNGTI<br>IHVKGKHLCPSPFPGPSKPFVWL VV VGGVLACYSLLVTVAFIIFWVRSKRSRL<br>LHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ<br>NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM<br>AEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                              |
| 6          | CAR25         | DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKWIYATSNL<br>ASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPTFGGGTKLEIK<br>GSTSGGGSGGGSGGGSGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTSY<br>NMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQ<br>LSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTTVTVSSESKYGPCCPPCPA<br>PEFDGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGV<br>EVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK<br>TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ<br>PENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT<br>QKSLSLSLGKMFVWL VV VGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFM<br>RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM<br>KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR |

FIG. 2B

| SEQ ID NO. | CAR construct | Amino acid sequence (Mature CAR)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 76         | CAR7          | DIVMTQTPHSSPVTLGQPASISCRSSQSLVSRDGNTYLSWLQQRPGQPPRLLIY<br>KISNRFSGVPNRFSGSGAGTDFTLKISRVKAEDVGYYCMQATQFPLTFGQGT<br>RLEIKGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYW<br>IGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQGQVTISADKSISTAYLQWSSL<br>KASDTAMYYCARQGD FWSGYGGMDVWGQGT TTVTVSSIEVMYPPPYLDNEK<br>SNGTIIHVKGKHLCPSP LFPGPSKPFWVLVVGGVLACYSLLVTVAFIIFWVRSK<br>RSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQ<br>QGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKD<br>KMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                      |
| 77         | CAR22         | DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWYATSNL<br>ASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPTFGGGTKLEIKG<br>STSGGGSGGGGSGGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNM<br>HWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLT<br>SEDSADYYCARSNYGSSYWFFDVWGAGTTTVTVSSLDESKYGPPCPPCPAPEF<br>LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHN<br>AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYCKVSNKGLPSSIEKTISKAK<br>GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK<br>TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL<br>GKMFVVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGGHSDYMNMTPRRPG<br>PTRKHYPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE<br>EEEGGCELRVKFSRSADAPAYQGQNQLYNELNLGRREEYDVL DKRRGRDPE<br>MGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGK GHDGLYQGLST<br>ATKDTYDALHMQALPPR |

FIG. 2B (cont'd)

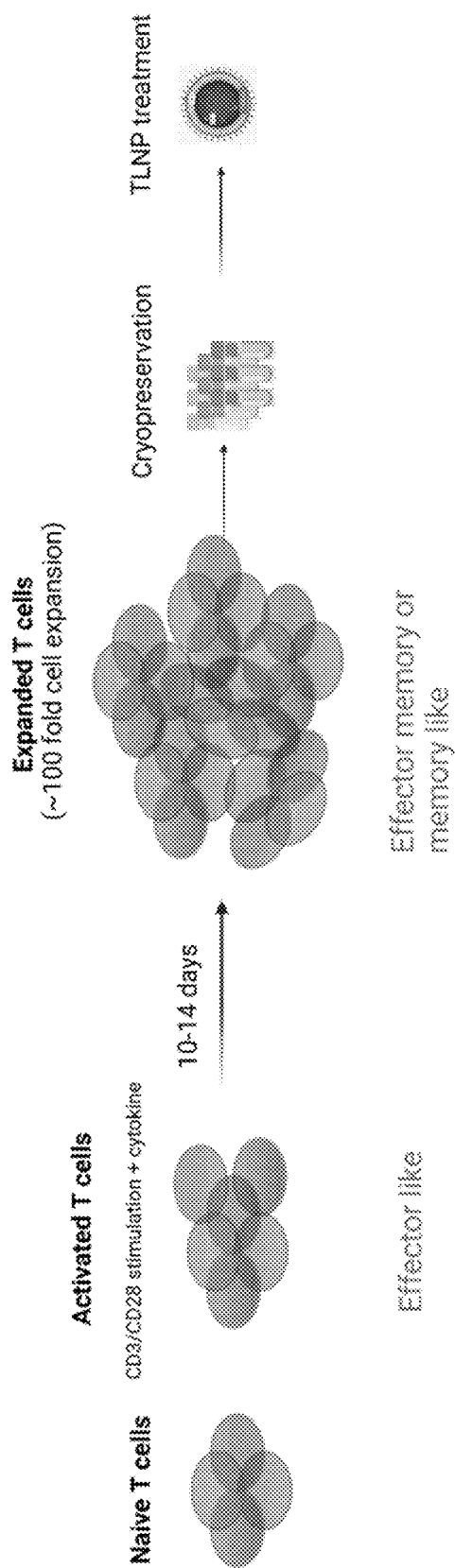
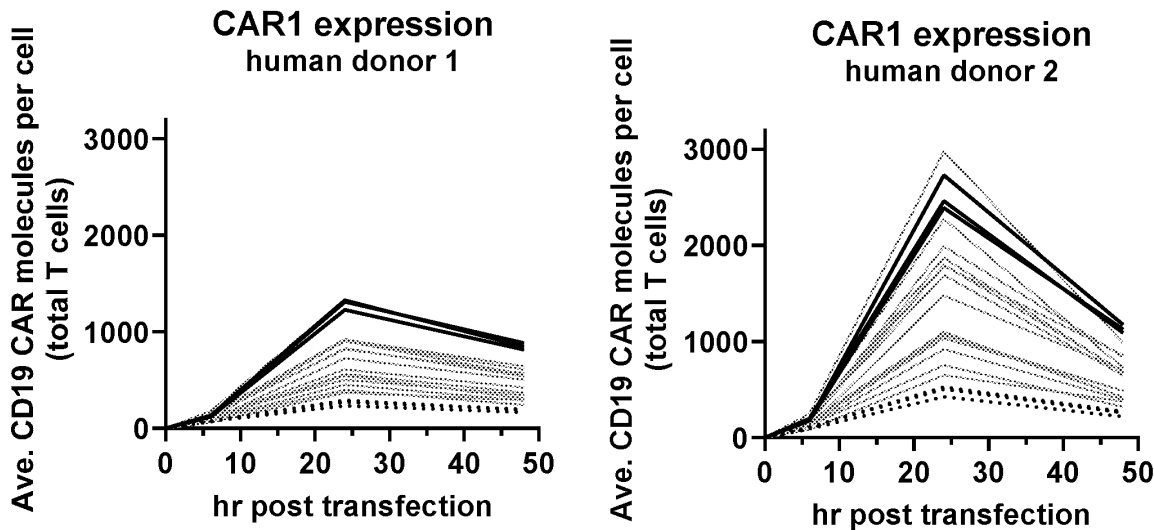


FIG. 3A

Expanded T cells

CAR1



CAR2

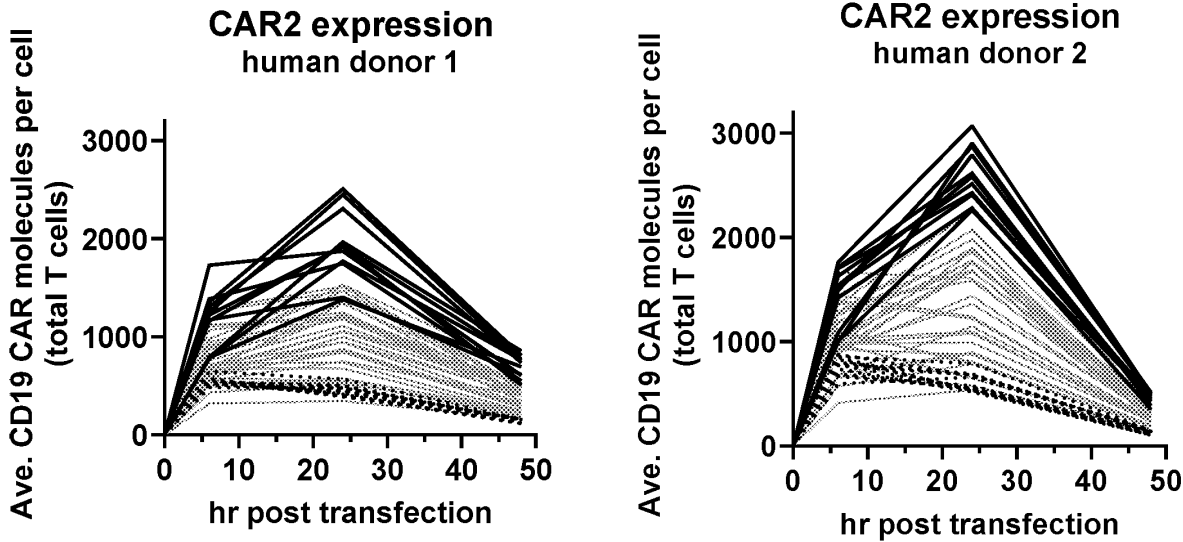


FIG. 3B

| SEQ ID NO: | RM       | 5' UTR           | ORF encoded CAR-Codon optimization strategy (OPT) | 3' UTR             | Round 2 Tier | Round 3 Tier |
|------------|----------|------------------|---------------------------------------------------|--------------------|--------------|--------------|
| 111        | RM_61117 | TEV              | CAR1-OPT6 single stop                             | XBG (no BsrGI)     | 3            | 3            |
| 112        | RM_61118 | TEV              | CAR2-OPT6 single stop                             | XBG (no BsrGI)     | 3            | 3            |
| 113        | RM_61321 | mHBB             | CAR1-OPT4                                         | PNLIP              | 1            | 1            |
| 114        | RM_61324 | mHBB             | CAR1-OPT4                                         | RPS3A              | 1            | 1            |
| 115        | RM_61326 | mHBB             | CAR1-OPT4                                         | RPS3A-PNLIP        | 1            | 1            |
| 116        | RM_61329 | CPA1             | CAR1-OPT4                                         | CPA1               | 2            | 2            |
| 117        | RM_61330 | DEFA3            | CAR1-OPT4                                         | DEFA3              | 2            | 1            |
| 118        | RM_61334 | CD8a             | CAR1-OPT4                                         | hHBA1              | 3            | 3            |
| 119        | RM_61340 | synthetic        | CAR1-OPT4                                         | hHBA1              | 2            | 2            |
| 120        | RM_61341 | HBA              | CAR1-OPT4                                         | AES-mtRNR1         | 2            | 2            |
| 121        | RM_61347 | mHBB             | CAR1-OPT4                                         | hHBA1              | 2            | 1            |
| 122        | RM_61348 | hAlb             | CAR1-OPT4                                         | hHBA1              | 2            | 2            |
| 123        | RM_61349 | mHBB             | CAR1-OPT4                                         | hHBA1-3x miR122 bs | 1            | 1            |
| 124        | RM_61350 | TEV              | CAR1-OPT7                                         | XBG                | 2            | 1            |
| 125        | RM_61351 | TEV              | CAR1-OPT5                                         | XBG                | 2            | 2            |
| 126        | RM_61352 | TEV              | CAR1-OPT3                                         | XBG                | 3            | 3            |
| 127        | RM_61353 | TEV              | CAR1-OPT2                                         | XBG                | 3            | 2            |
| 128        | RM_61355 | mHBB             | CAR2-OPT4                                         | hHBA1              | 1            | 1            |
| 129        | RM_61356 | hAlb             | CAR2-OPT4                                         | hHBA1              | 2            | 1            |
| 130        | RM_61357 | mHBB             | CAR2-OPT4                                         | hHBA1-3x miR122 bs | 1            | 1            |
| 131        | RM_61358 | TEV              | CAR2-OPT7                                         | XBG                | 2            | 1            |
| 132        | RM_61359 | TEV              | CAR1-OPT4                                         | XBG                | 2            | 2            |
| 133        | RM_61360 | TEV              | CAR2-OPT4                                         | XBG                | 2            | 2            |
| 134        | RM_61362 | TEV              | CAR1-OPT6 double stop                             | XBG                | 3            | 3            |
| 135        | RM_61363 | TEV              | CAR2-OPT6 triple stops                            | XBG                | 3            | 3            |
| 136        | RM_61378 | eIF4G aptamer x1 | CAR1-OPT4                                         | hHBA1              | 2            | 1            |
| 137        | RM_61379 | aptamer control  | CAR1-OPT4                                         | hHBA1              | 2            | 1            |
| 138        | RM_61411 | TEV              | CAR2-OPT1                                         | XBG                | 2            | 2            |

FIG. 3C

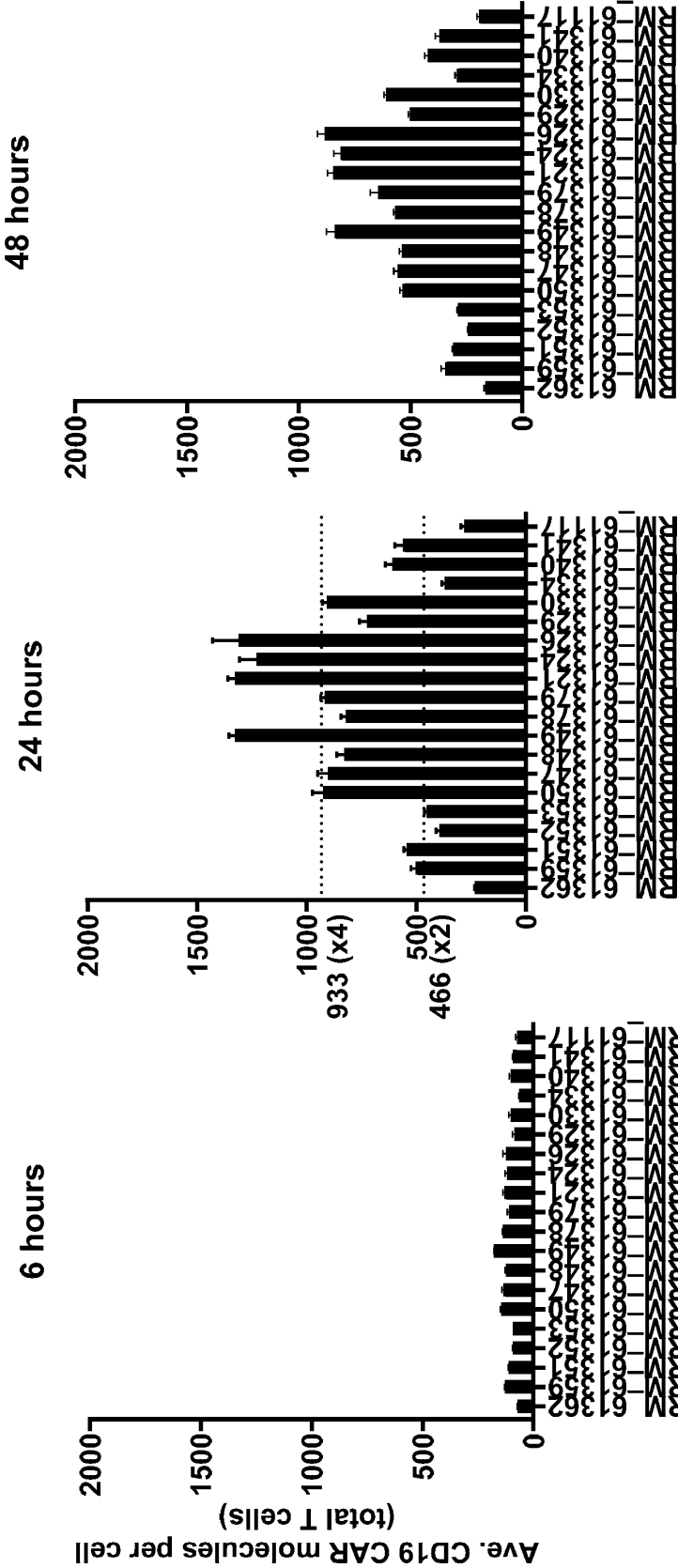
| SEQ ID NO: | RM       | 5' UTR           | ORF encoded CAR-Codon optimization strategy (OPT) | 3' UTR      | Round 2 Tier | Round 3 Tier |
|------------|----------|------------------|---------------------------------------------------|-------------|--------------|--------------|
| 139        | RM_61449 | TEV              | CAR2-OPT2                                         | XBG         | 2            | 3            |
| 140        | RM_61450 | TEV              | CAR2-OPT3                                         | XBG         | 2            | 3            |
| 141        | RM_61451 | mHBB             | CAR2-OPT2                                         | hHBA1       | 2            | 2            |
| 142        | RM_61452 | mHBB             | CAR2-OPT3                                         | hHBA1       | 2            | 2            |
| 143        | RM_61453 | synthetic        | CAR2-OPT4                                         | hHBA1       | 2            | 2            |
| 144        | RM_61454 | HBA              | CAR2-OPT4                                         | AES-mtRNR1  | 2            | 2            |
| 145        | RM_61455 | DEFA3            | CAR2-OPT4                                         | DEFA3       | 2            | 1            |
| 146        | RM_61456 | CPA1             | CAR2-OPT4                                         | CPA1        | 2            | 2            |
| 147        | RM_61457 | CD8a             | CAR2-OPT4                                         | hHBA1       | 3            | 2            |
| 148        | RM_61458 | eIF4G aptamer x1 | CAR2-OPT4                                         | hHBA1       | 2            | 1            |
| 149        | RM_61459 | aptamer control  | CAR2-OPT4                                         | hHBA1       | 2            | 2            |
| 150        | RM_61460 | mHBB             | CAR2-OPT4                                         | RPS3A       | 2            | 2            |
| 151        | RM_61461 | mHBB             | CAR2-OPT4                                         | PNLIP-RPS3A | 1            | 1            |
| 152        | RM_61462 | DEFA3            | CAR2-OPT2                                         | DEFA3       | 2            | 2            |
| 153        | RM_61463 | CPA1             | CAR2-OPT2                                         | CPA1        | 2            | 3            |
| 154        | RM_61464 | CD8a             | CAR2-OPT2                                         | hHBA1       | 3            | 3            |
| 155        | RM_61465 | eIF4G aptamer x1 | CAR2-OPT2                                         | hHBA1       | 2            | 2            |
| 156        | RM_61466 | aptamer control  | CAR2-OPT2                                         | hHBA1       | 2            | 2            |
| 157        | RM_61467 | mHBB             | CAR2-OPT2                                         | RPS3A       | 2            | 2            |
| 158        | RM_61468 | mHBB             | CAR2-OPT2                                         | PNLIP-RPS3A | 2            | 2            |
| 159        | RM_61482 | mHBB             | CAR2-OPT1                                         | hHBA1       | 1            | 1            |
| 160        | RM_61483 | DEFA3            | CAR2-OPT1                                         | DEFA3       | 1            | 1            |
| 161        | RM_61484 | CPA1             | CAR2-OPT1                                         | CPA1        | 2            | 2            |
| 162        | RM_61485 | CD8a             | CAR2-OPT1                                         | hHBA1       | 2            | 2            |
| 163        | RM_61486 | eIF4G aptamer x1 | CAR2-OPT1                                         | hHBA1       | 1            | 1            |
| 164        | RM_61487 | aptamer control  | CAR2-OPT1                                         | hHBA1       | 1            | 1            |

FIG. 3C (cont'ed)

| SEQ ID NO: | RM       | 5' UTR                 | ORF encoded CAR-Codon optimization strategy (OPT) | 3' UTR      | Round 2 Tier | Round 3 Tier |
|------------|----------|------------------------|---------------------------------------------------|-------------|--------------|--------------|
| 165        | RM_61488 | mHBB                   | CAR2-OPT1                                         | RPS3A       | 1            | 1            |
| 166        | RM_61489 | mHBB                   | CAR2-OPT1                                         | PNLIP-RPS3A | 1            | 1            |
| 167        | RM_61501 | TEV                    | CAR2-OPT5                                         | XBG         | 2            | 2            |
| 168        | RM_61512 | TEV                    | CAR2-OPT6<br>double stops                         | XBG         | 3            | 3            |
| 169        | RM_61513 | mHBB                   | CAR2-OPT6<br>double stops                         | hHBA1       | 3            | 3            |
| 170        | RM_61514 | DEFA3                  | CAR2-OPT6<br>double stops                         | DEFA3       | 3            | 3            |
| 171        | RM_61515 | CPA1                   | CAR2-OPT6<br>double stops                         | CPA1        | 2            | 2            |
| 172        | RM_61516 | CD8a                   | CAR2-OPT6<br>double stops                         | hHBA1       | 3            | 3            |
| 173        | RM_61517 | eIF4G<br>aptamer<br>x1 | CAR2-OPT6<br>double stops                         | hHBA1       | 3            | 3            |
| 174        | RM_61518 | aptamer<br>control     | CAR2-OPT6<br>double stops                         | hHBA1       | 3            | 3            |
| 175        | RM_61519 | mHBB                   | CAR2-OPT6<br>double stops                         | RPS3A       | 3            | 3            |
| 176        | RM_61520 | mHBB                   | CAR2-OPT6<br>double stops                         | PNLIP-RPS3A | 3            | 3            |

FIG. 3C (cont'ed)

CAR1 - Human Donor 1



**FIG. 31**

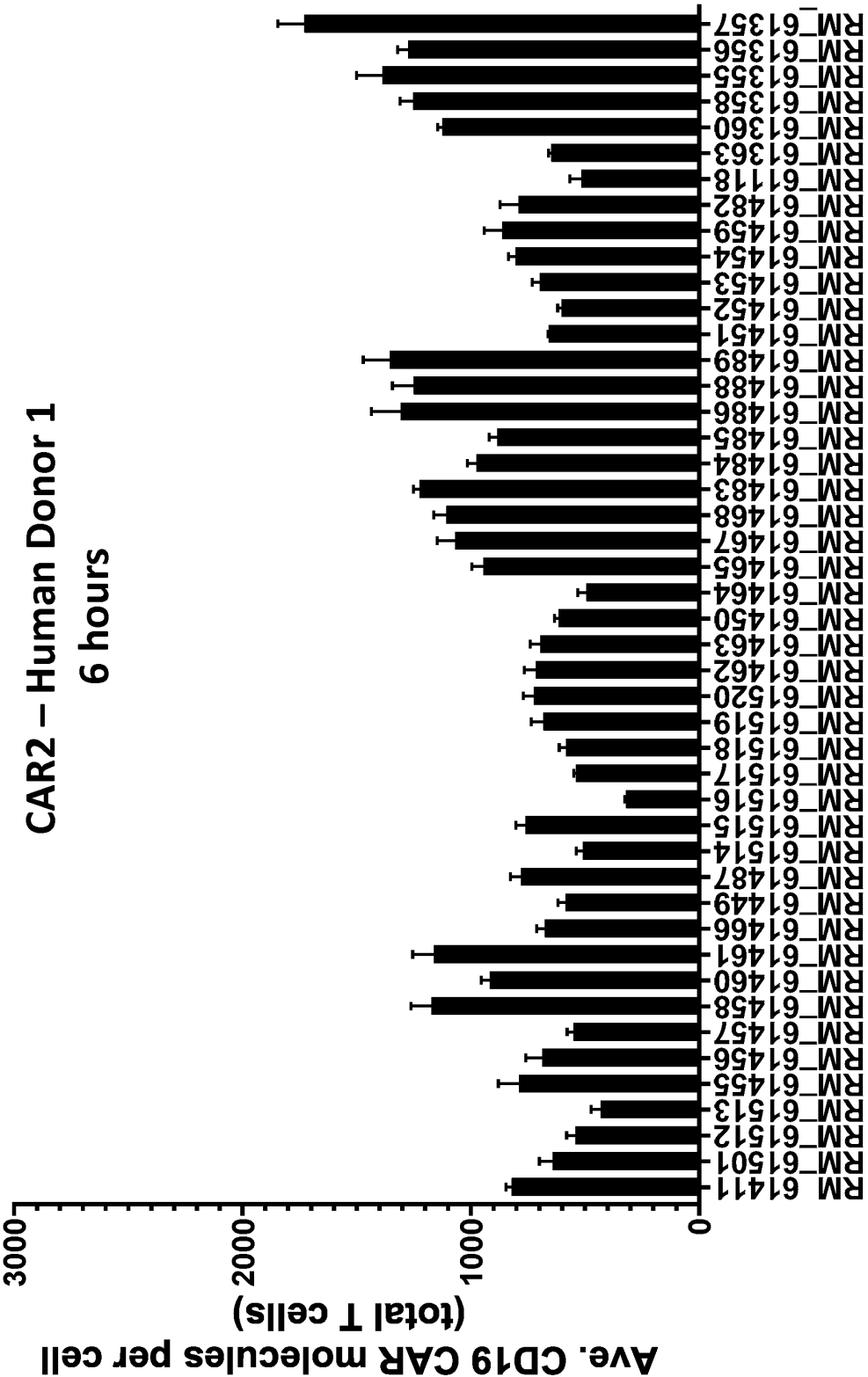


FIG. 3J

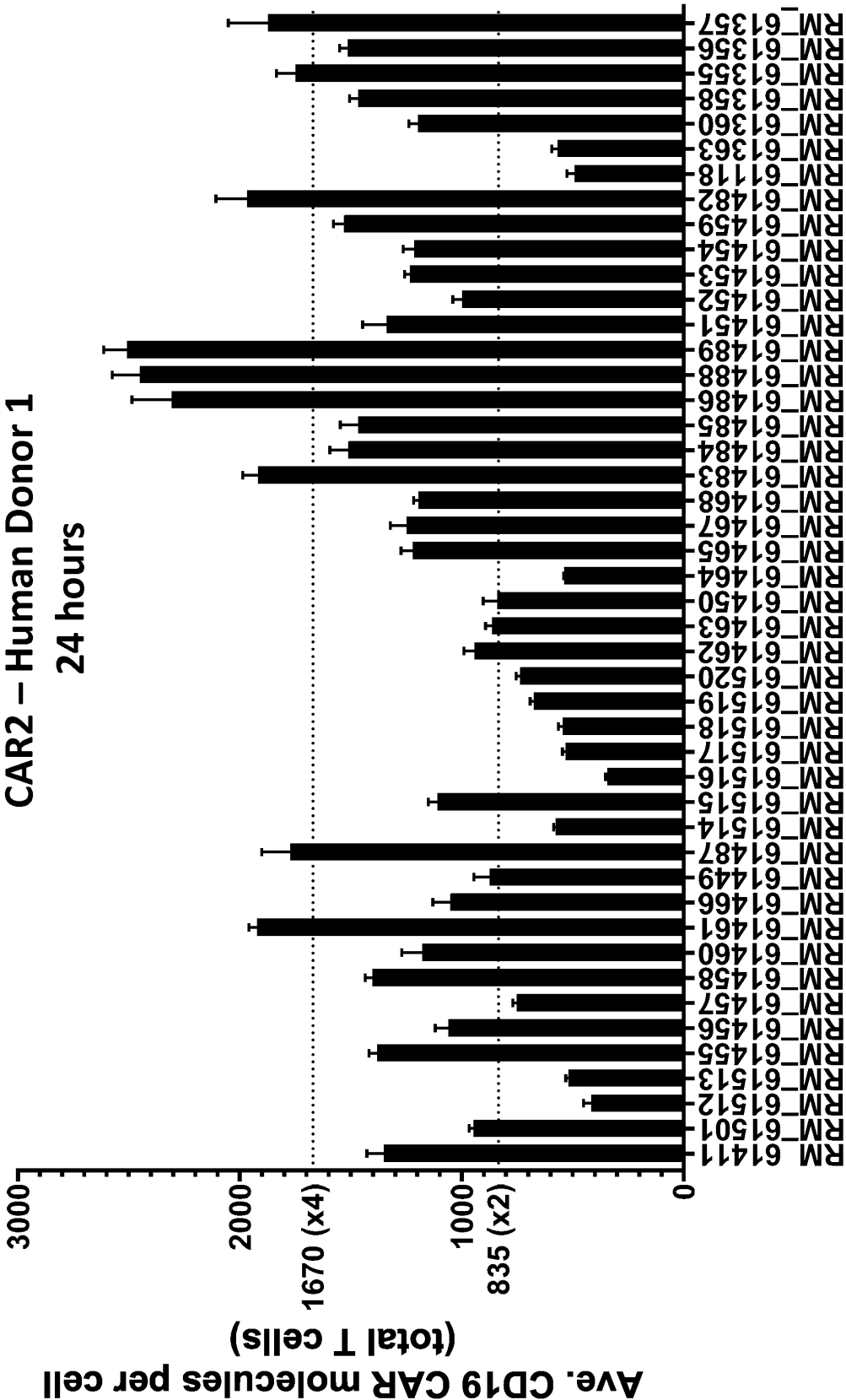


FIG. 3K

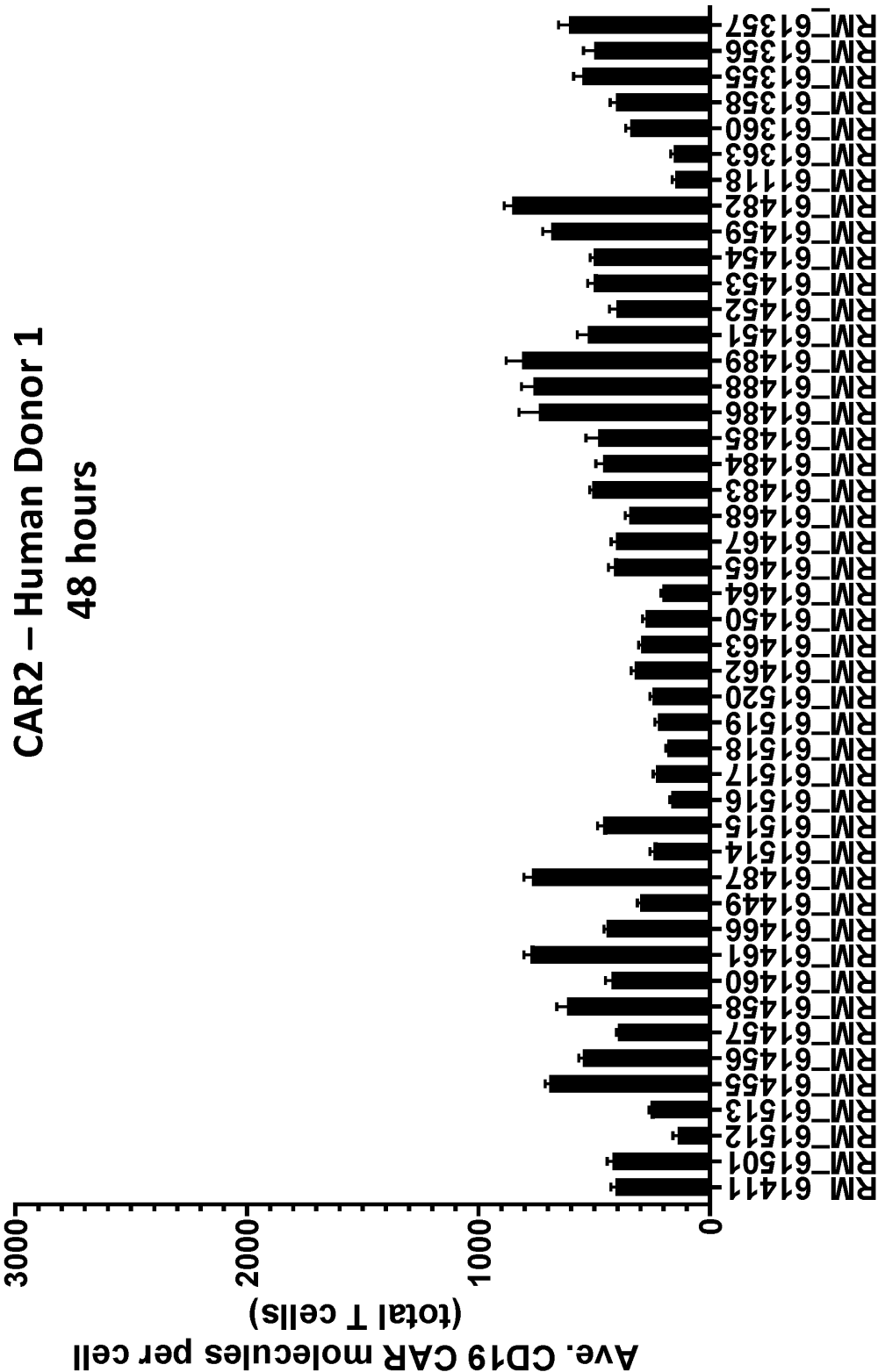


FIG. 3L

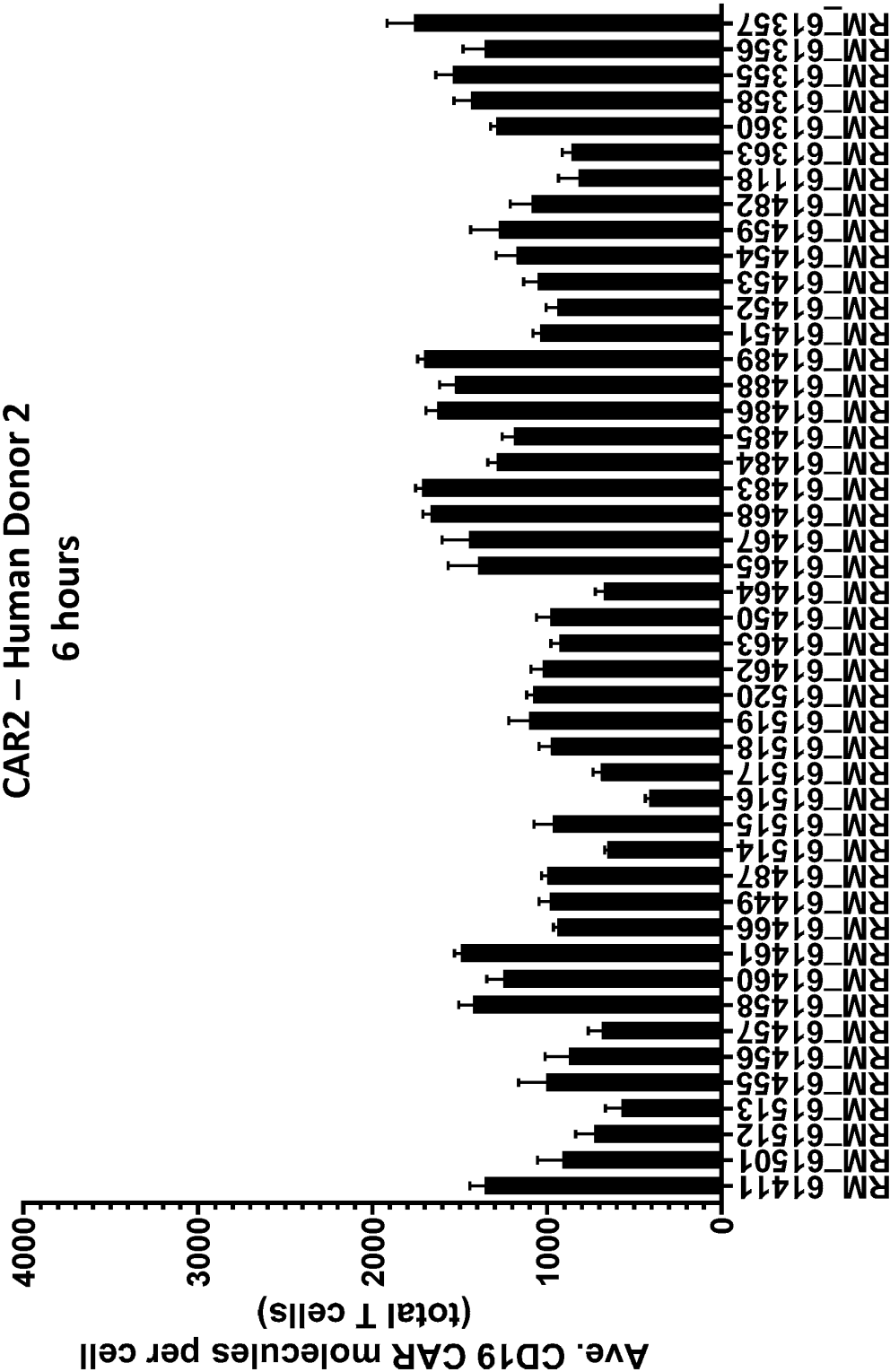


FIG. 3M

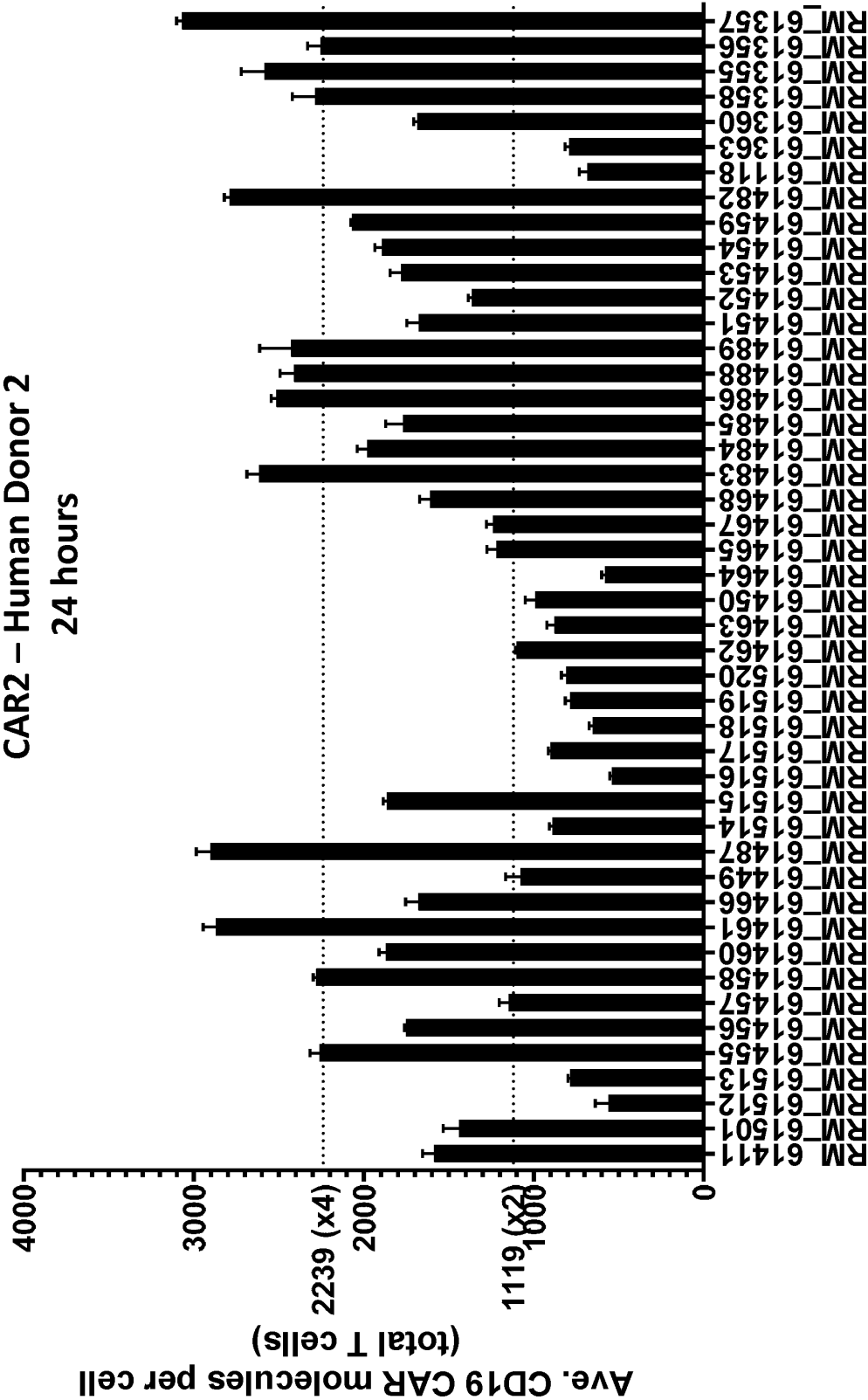


FIG. 3N

CAR2 – Human Donor 2  
48 hours



FIG. 30

## Expanded T cells from human donor 1

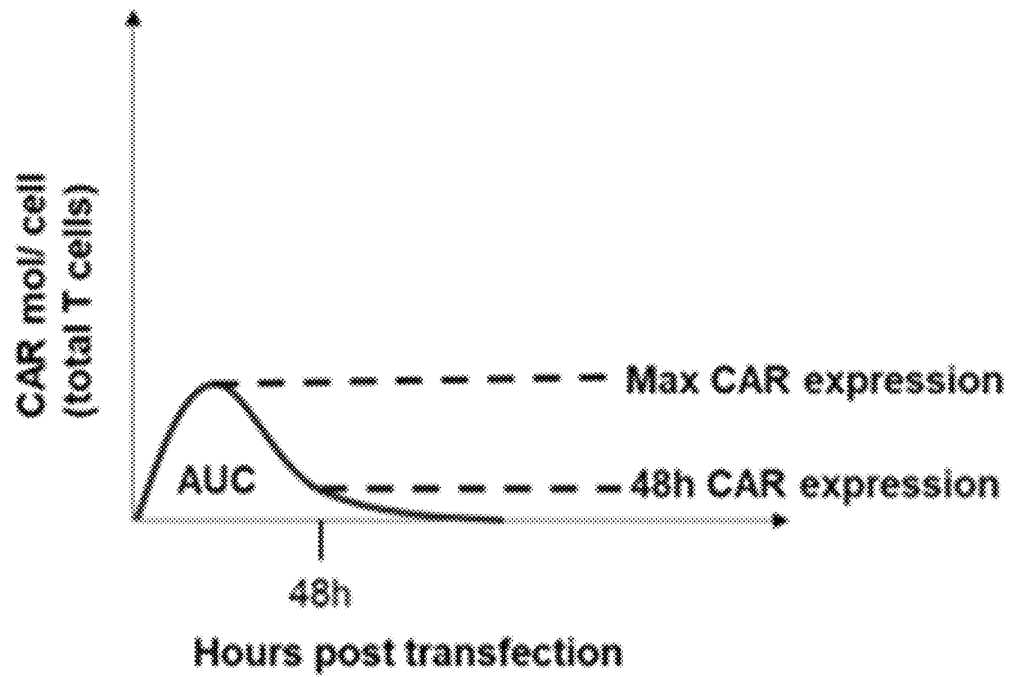


FIG. 4A

Expanded T cells from human donor 1

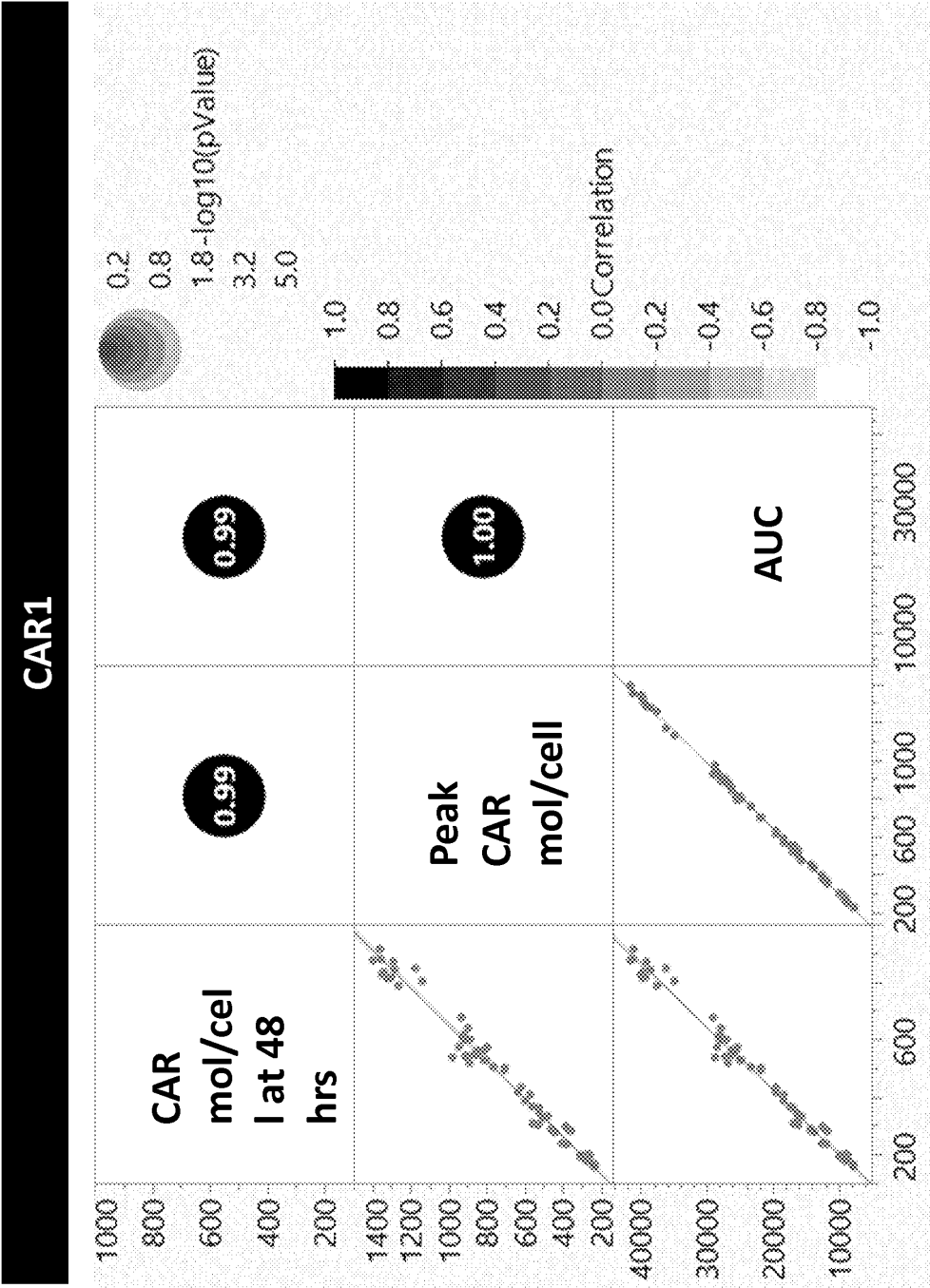
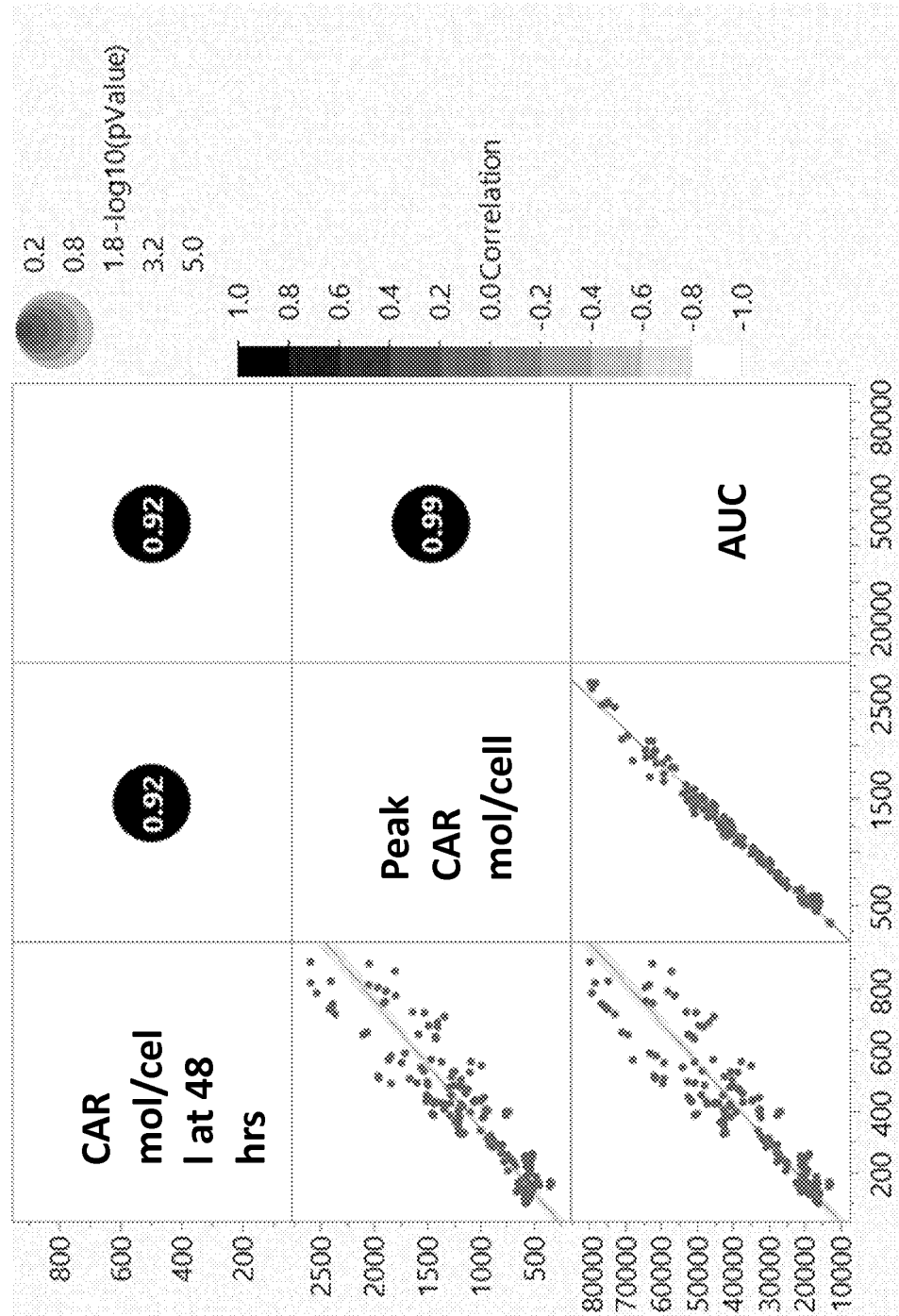


FIG. 4B

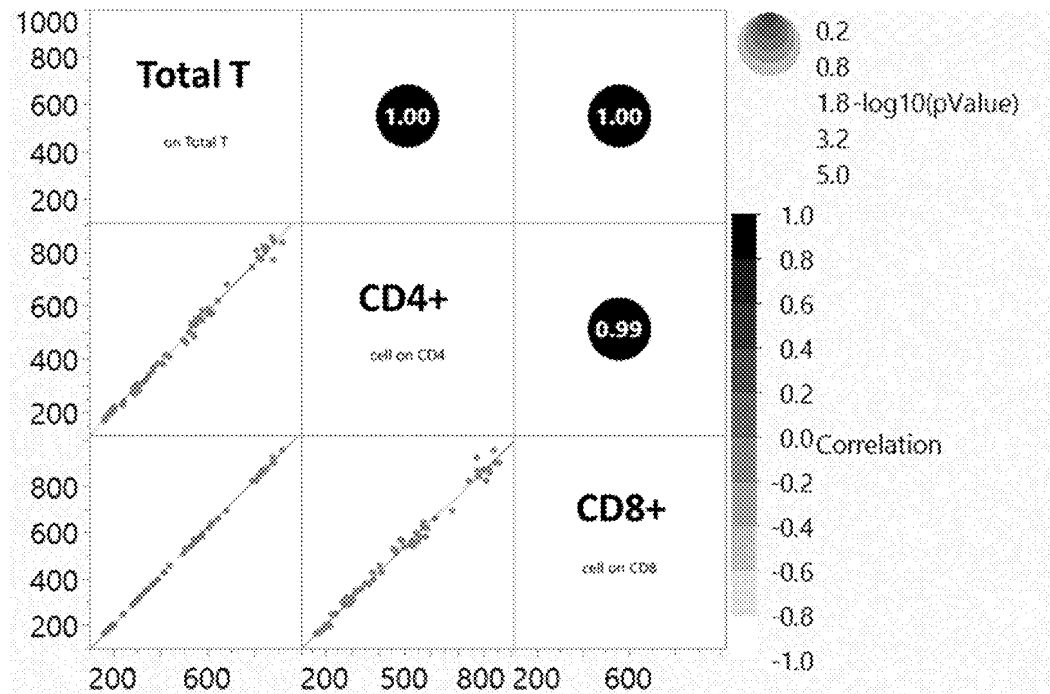
# CAR2



**FIG. 4C**

## Expanded T cells from human donor 1

## CAR1 mol/cell @48h



## CAR2 mol/cell @48h

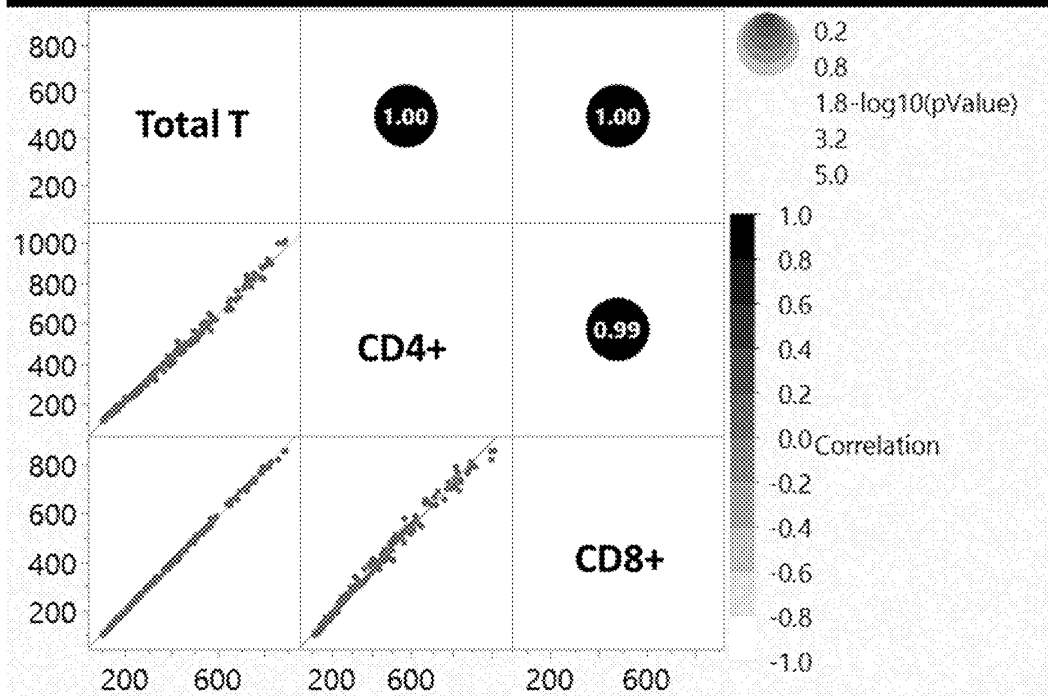
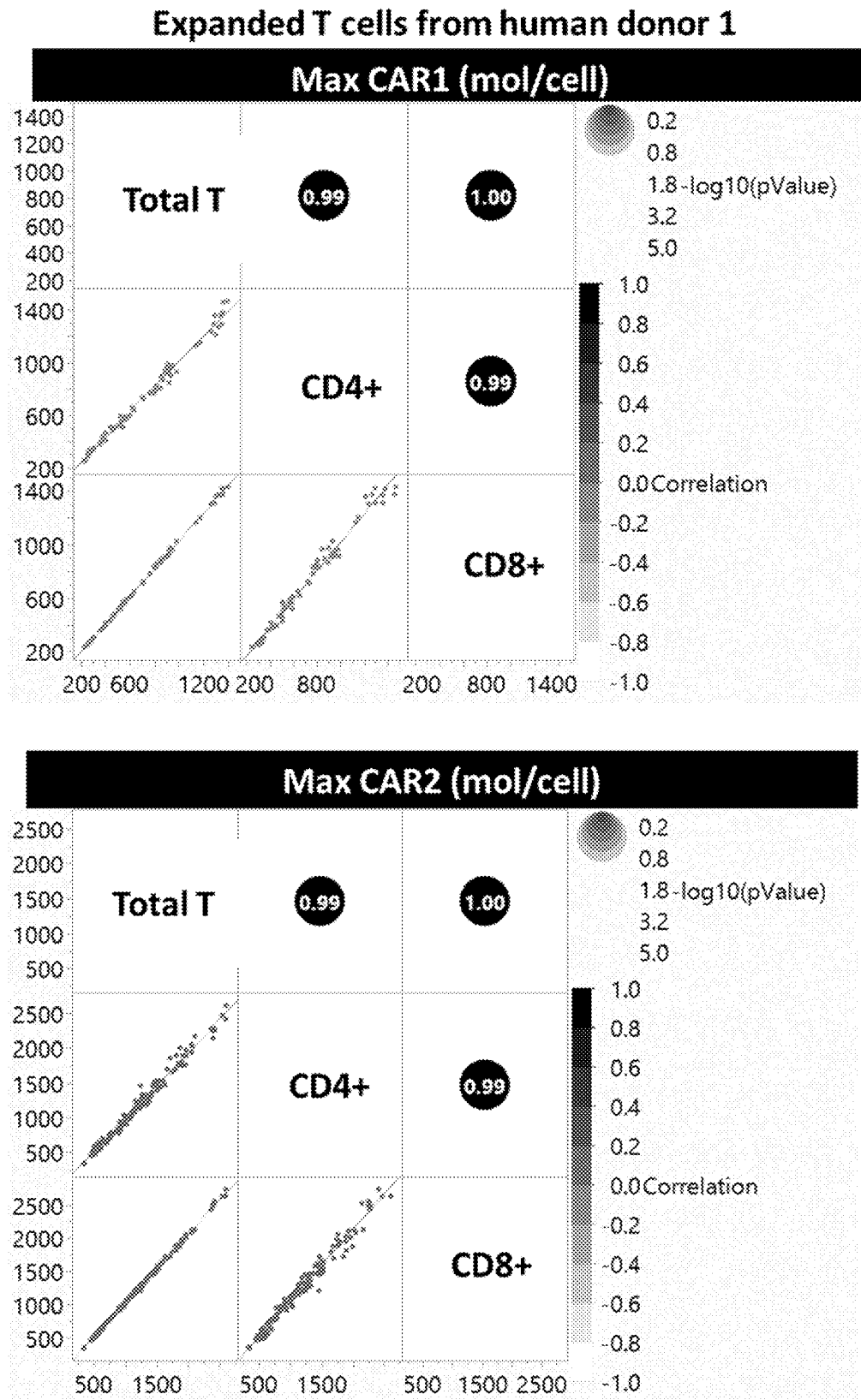


FIG. 4D

**FIG. 4E**

## Expanded T cells from human donor 1

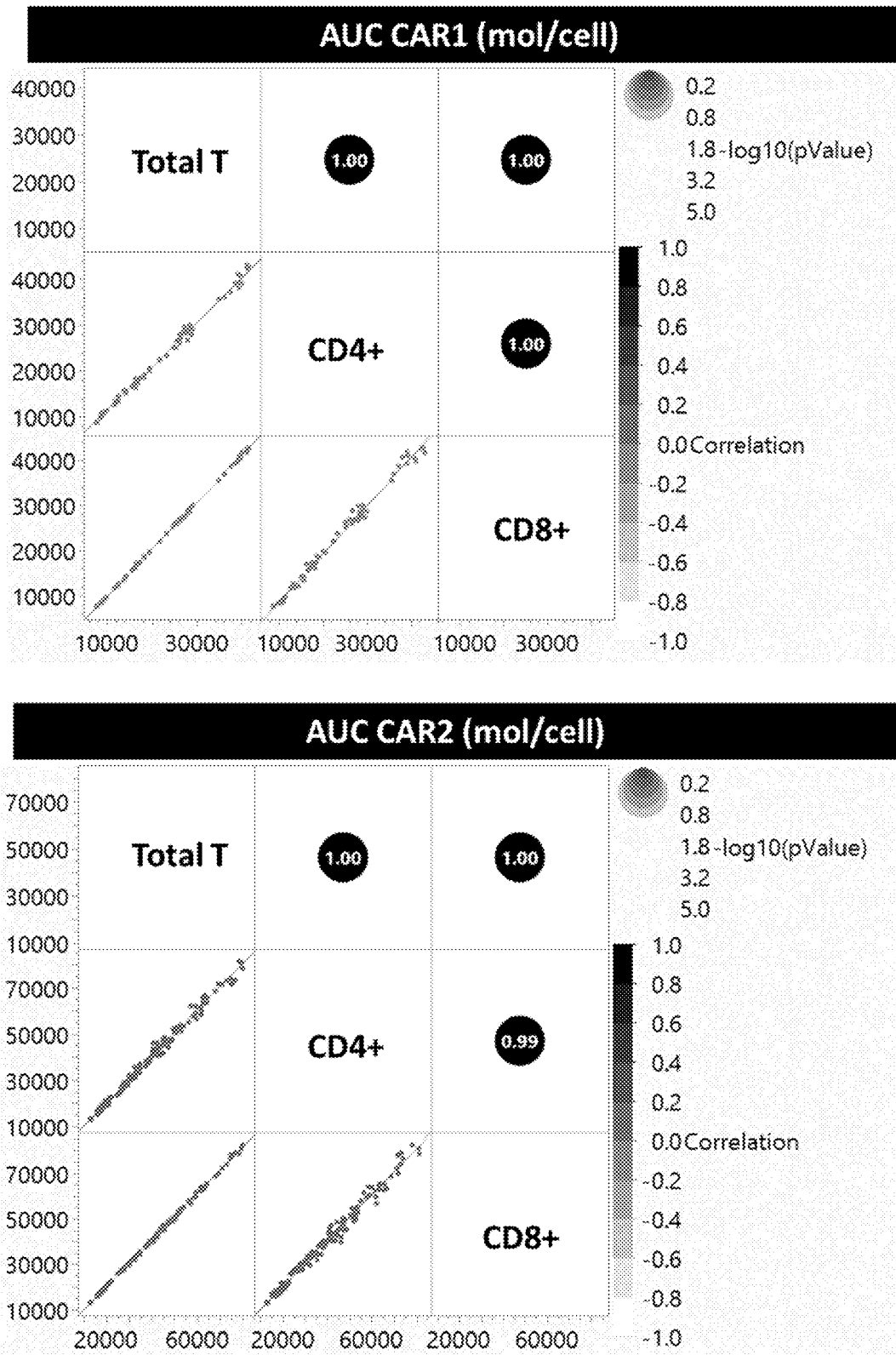


FIG. 4F

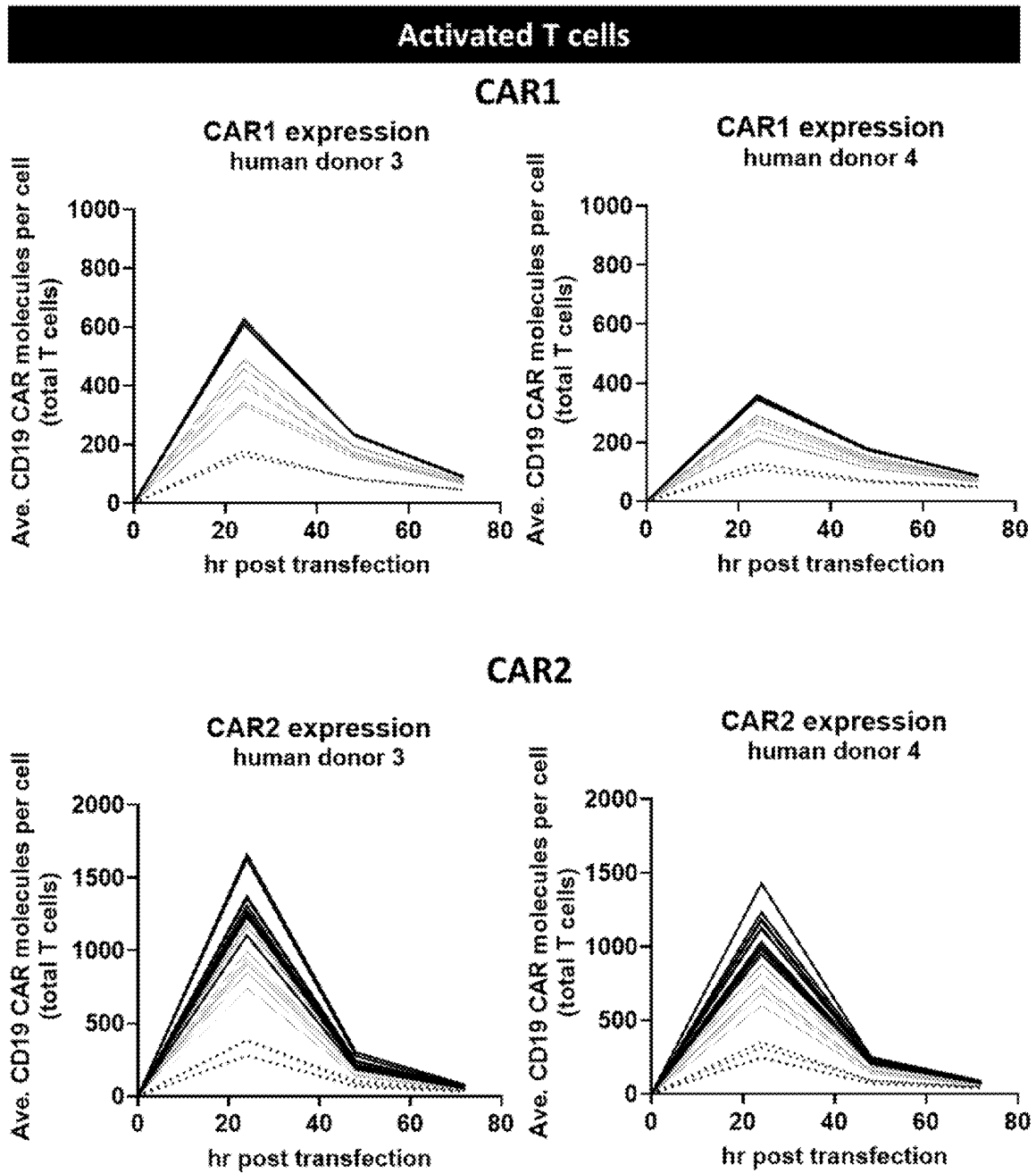


FIG. 5A

CAR1 – Human Donor 3

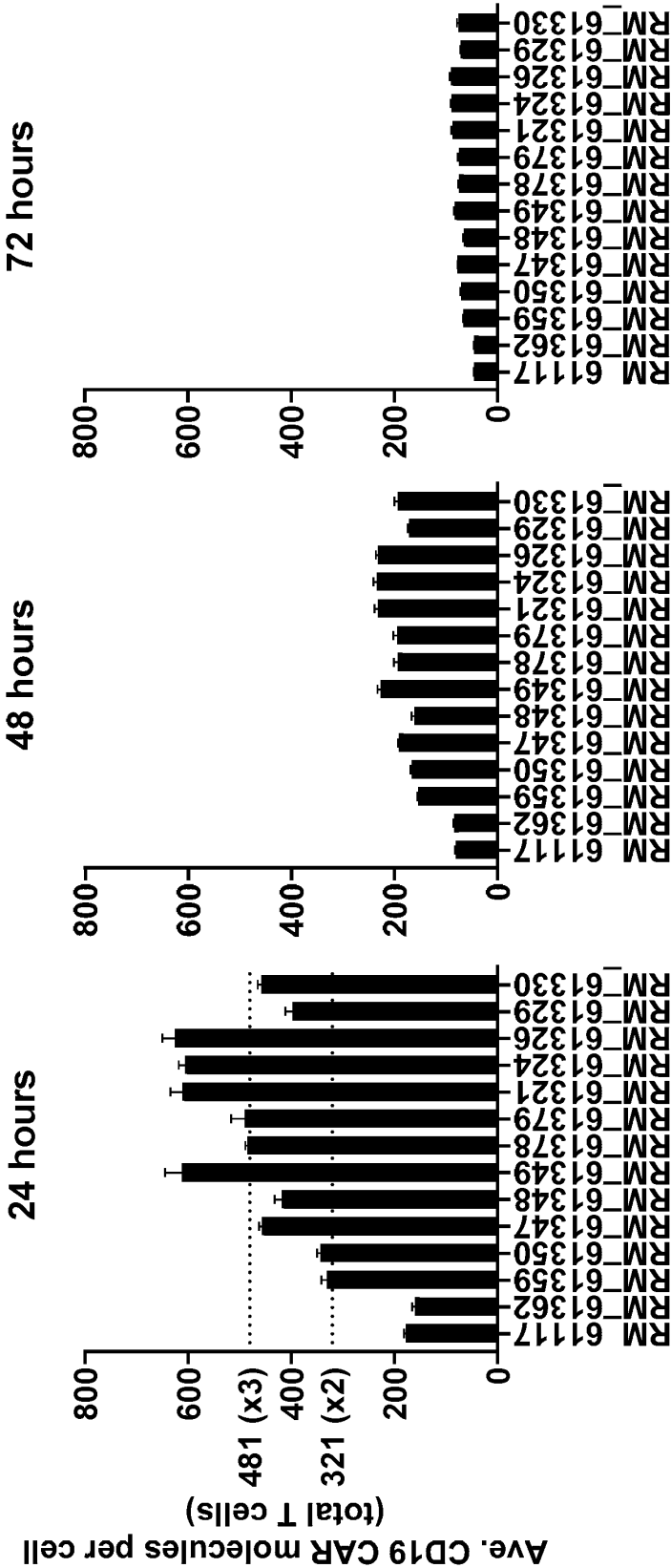


FIG. 5B

FIG. 5C

FIG. 5D

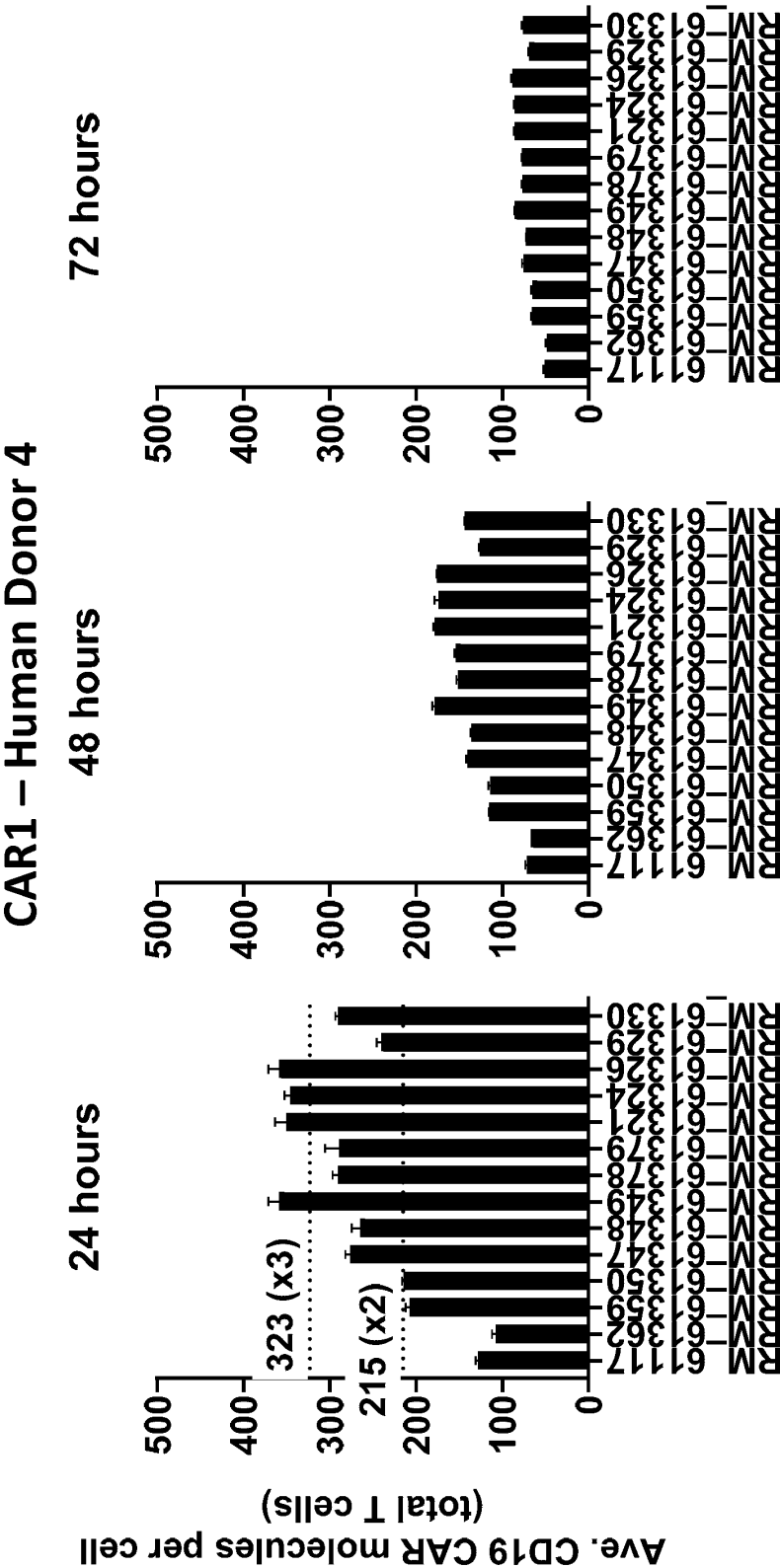


FIG. 5G

FIG. 5F

FIG. 5E

## CAR2 – Human Donor 3

24 hours

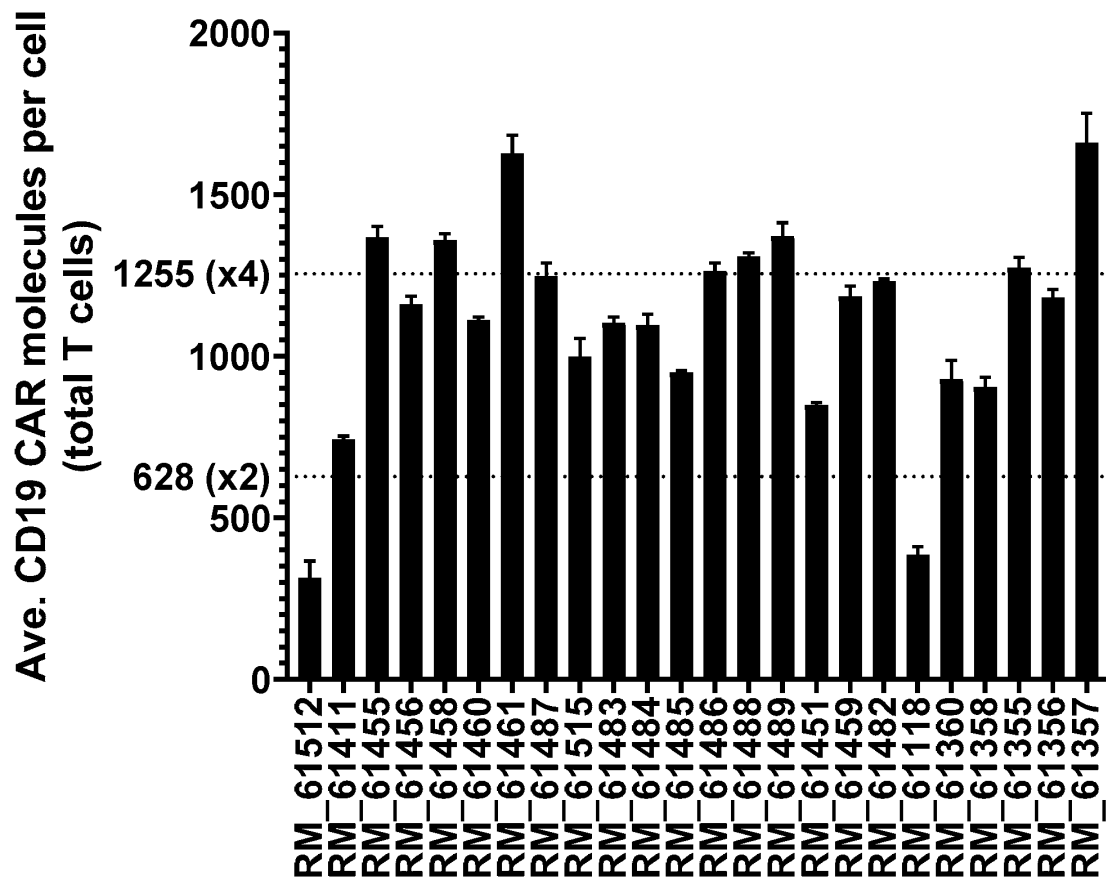


FIG. 5H

CAR2 – Human Donor 3

48 hours

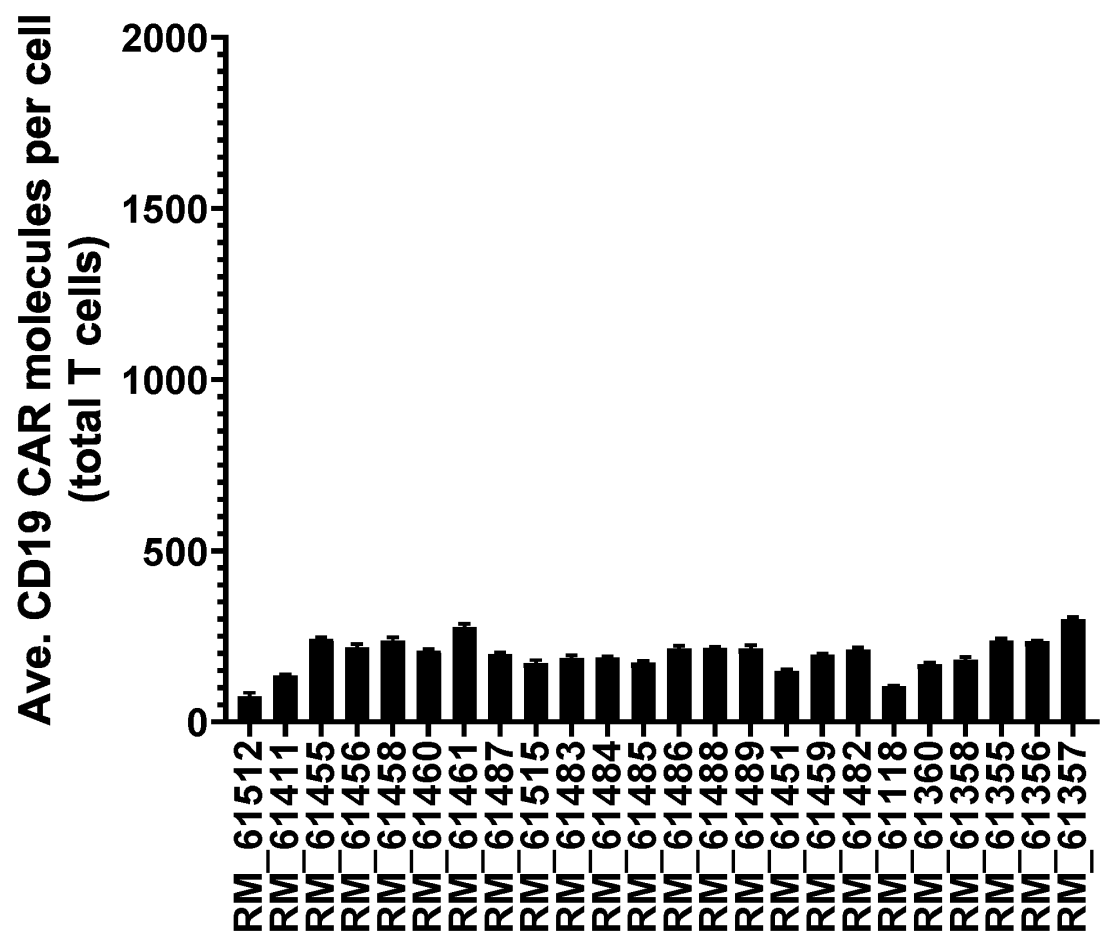


FIG. 5I

CAR2 – Human Donor 3  
72 hours

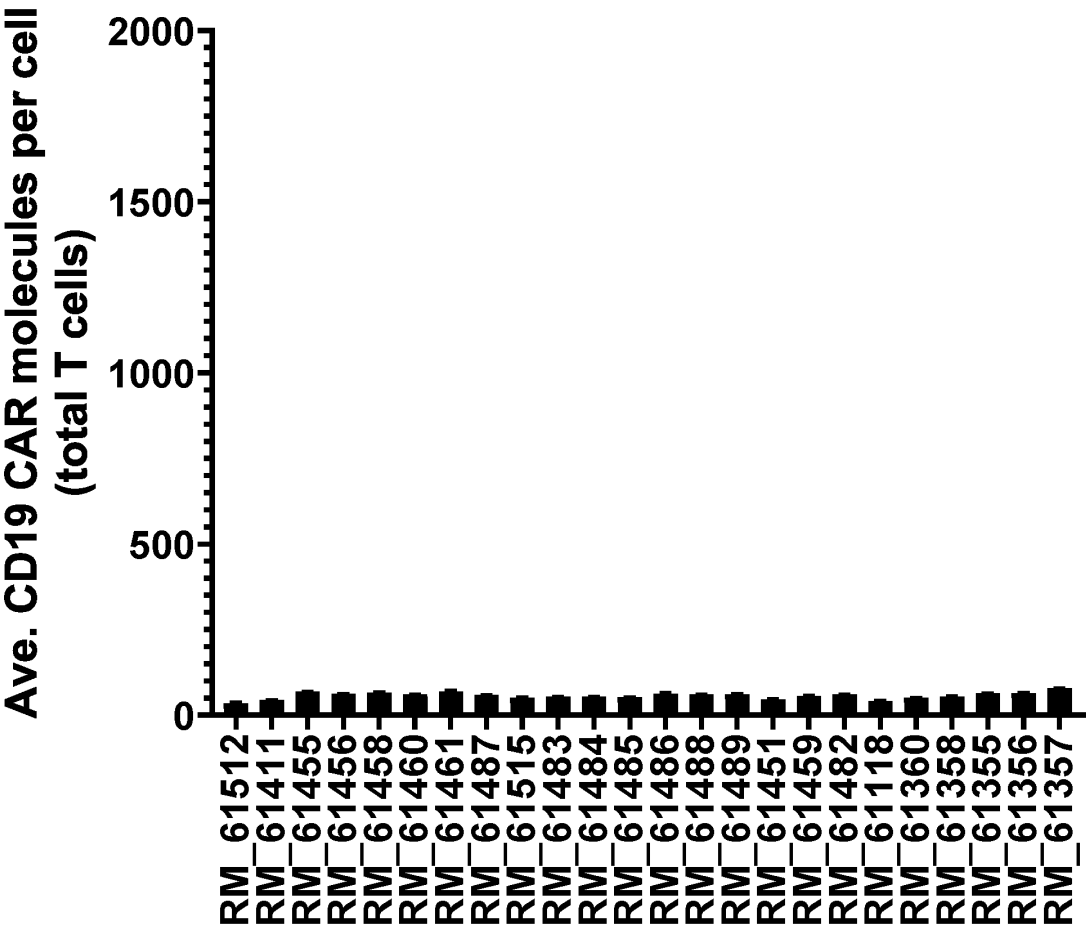


FIG. 5J

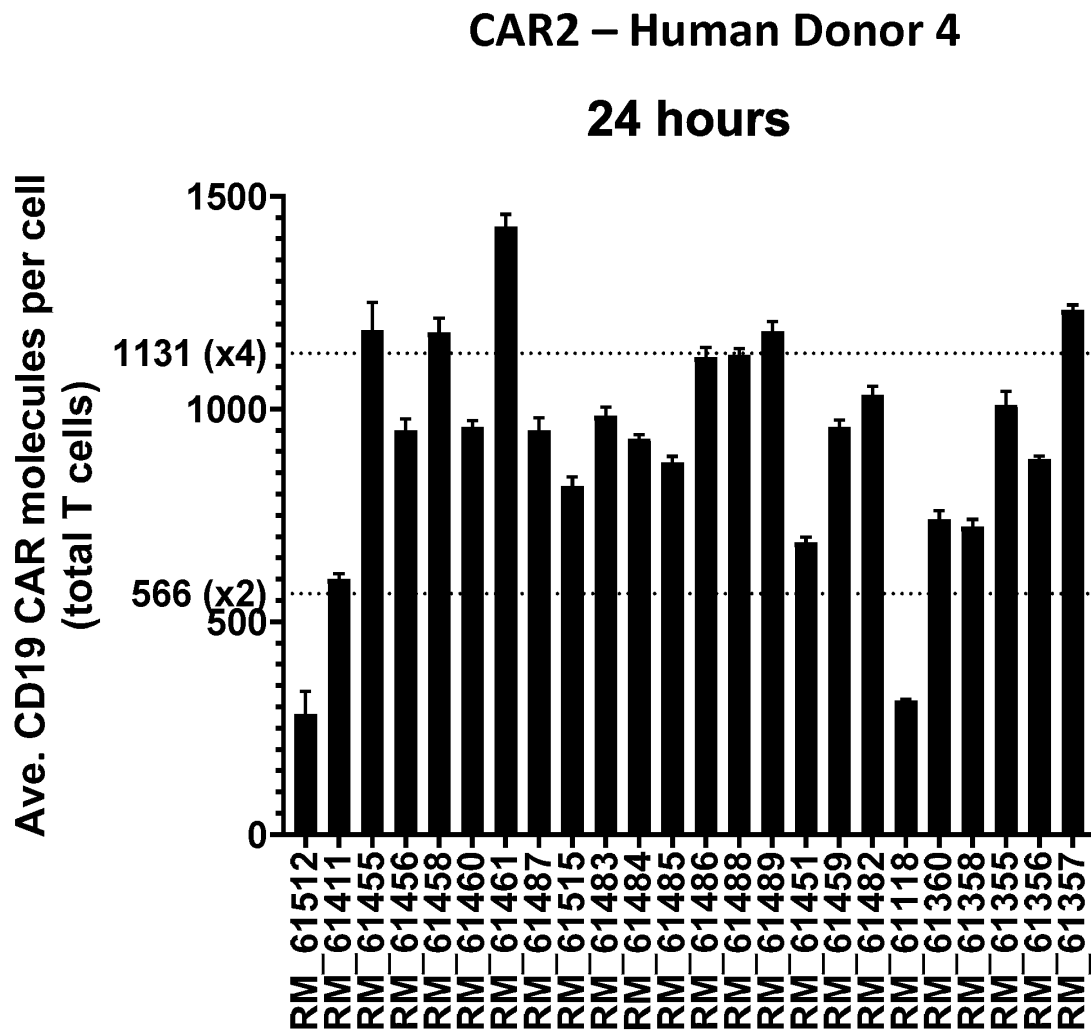


FIG. 5K

CAR2 – Human Donor 4

48 hours

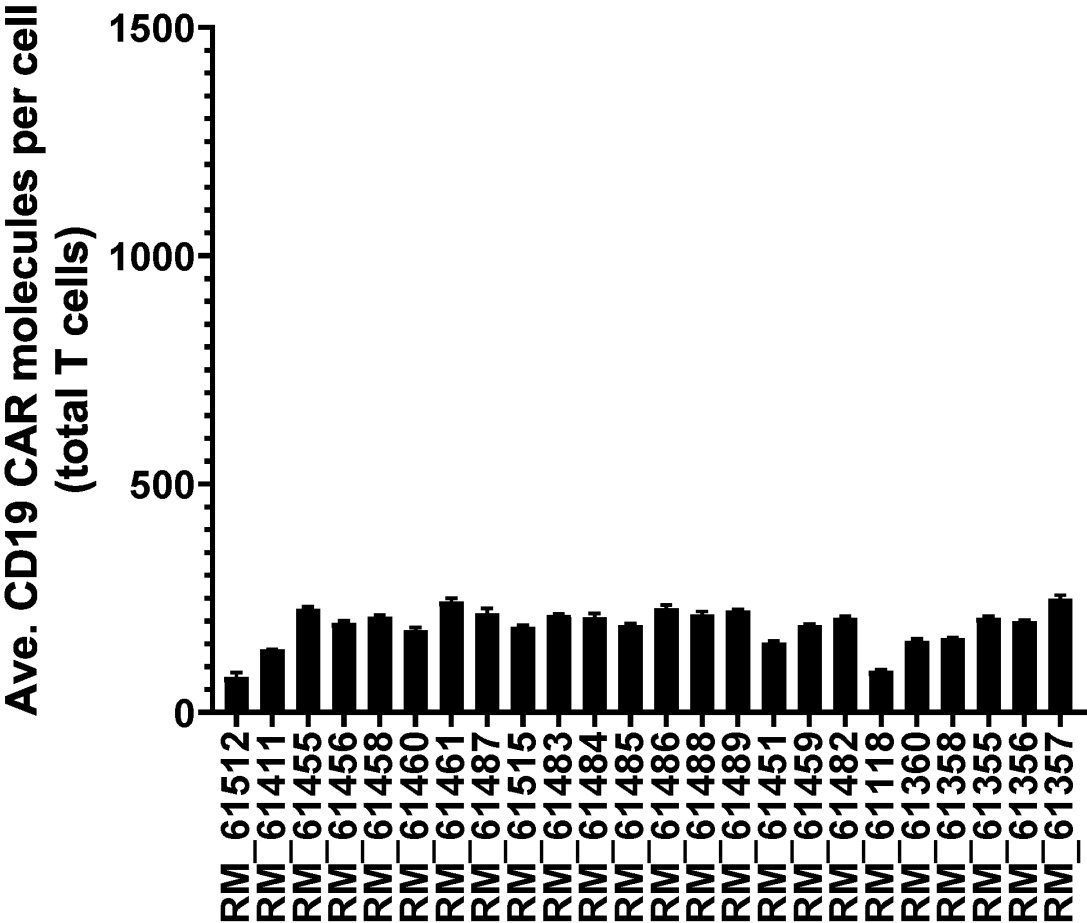


FIG. 5L

CAR2 – Human Donor 4

72 hours

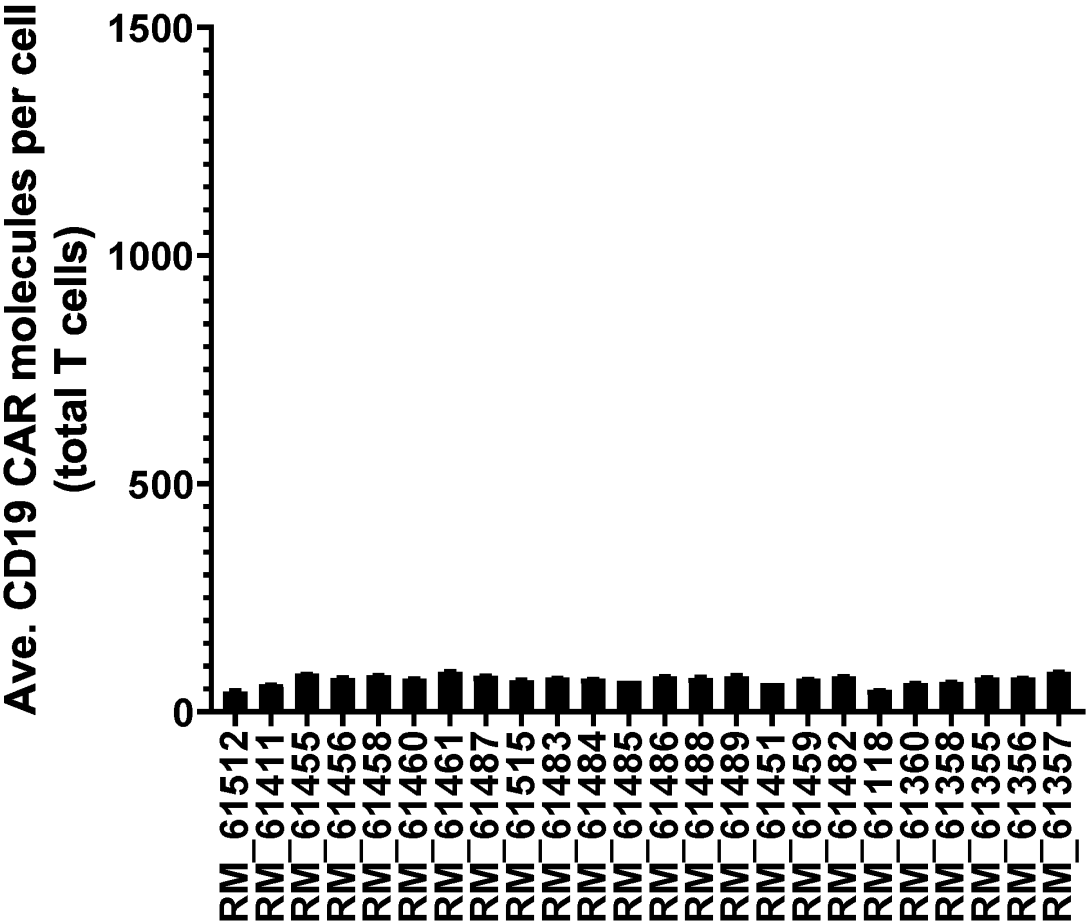
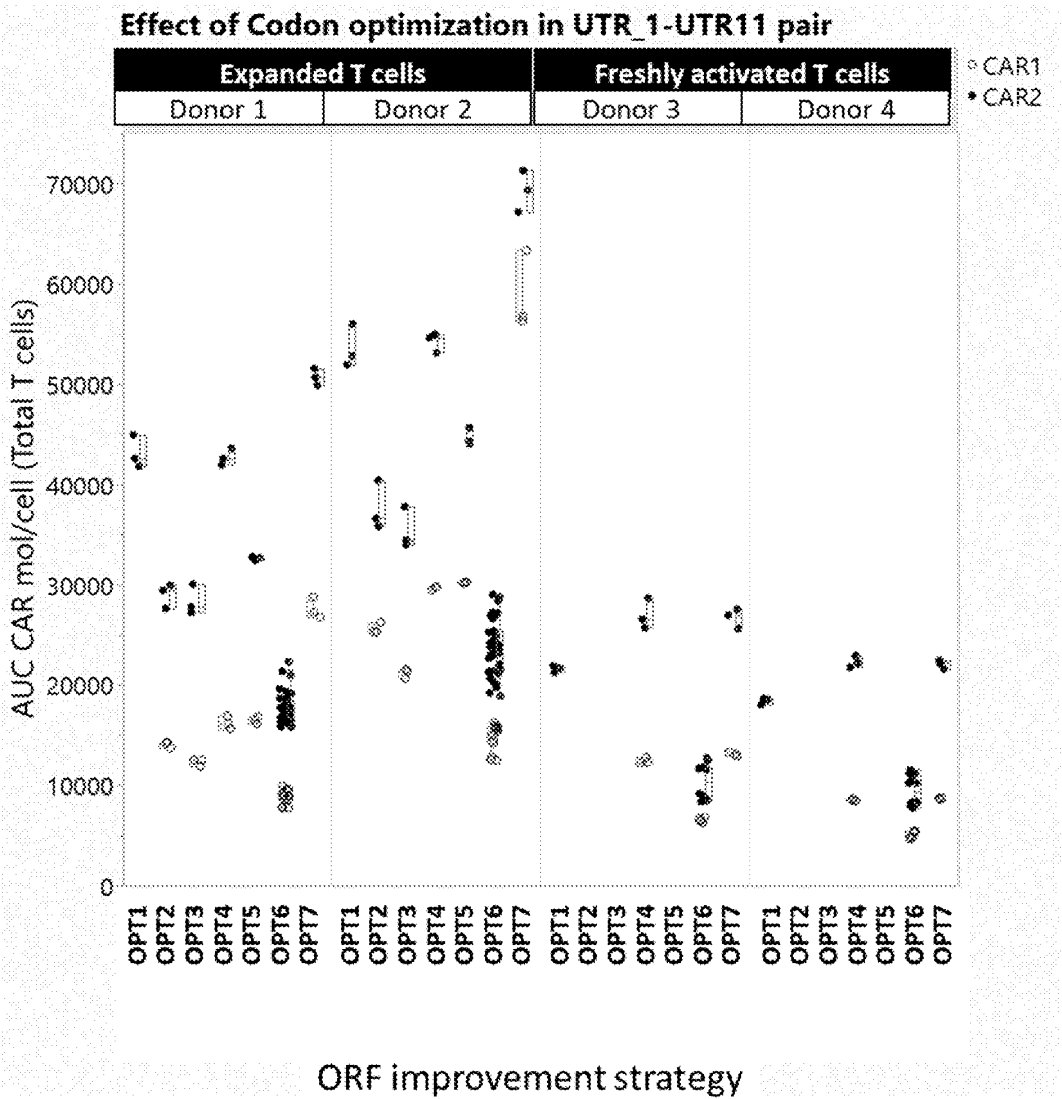


FIG. 5M



**FIG. 6**



| UTR combination | UTR combination code |
|-----------------|----------------------|
| TEV_XBG         | UTR_1 & UTR_11       |

FIG. 7

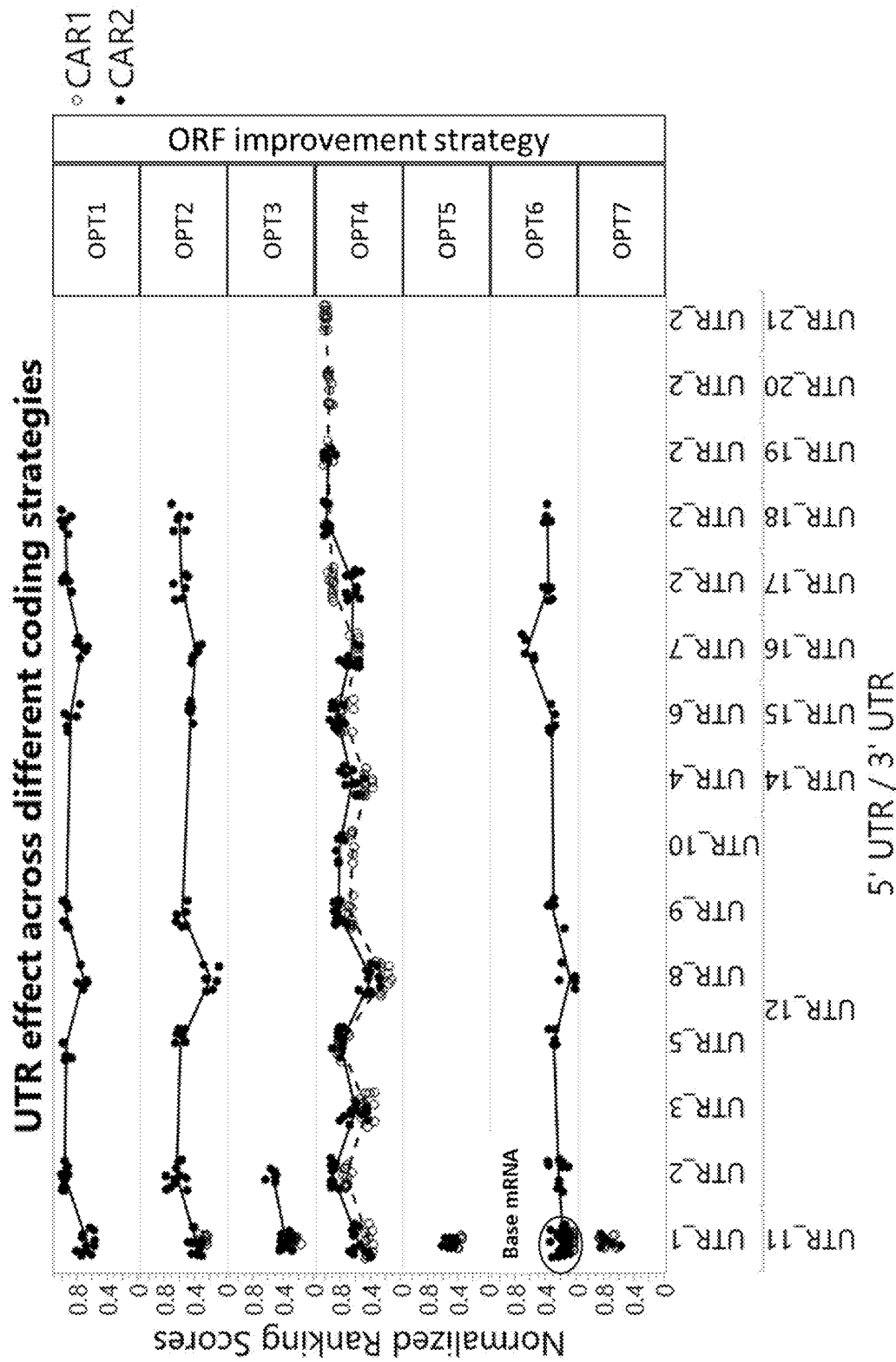


FIG. 8A

| UTR combination         | UTR combination code |
|-------------------------|----------------------|
| TEV_XBG                 | UTR_1 & UTR_11       |
| mHBB_PNLIP              | UTR_2 & UTR_20       |
| mHBB_RPS3A              | UTR_2 & UTR_17       |
| mHBB_RPS3A-PNLIP        | UTR_2 & UTR_21       |
| CPA1_CPA1               | UTR_7 & UTR_16       |
| DEFA3_DEFA3             | UTR_6 & UTR_15       |
| CD8a_hHBA1              | UTR_8 & UTR_12       |
| synthetic_hHBA1         | UTR_3 & UTR_12       |
| HBA_AES-mtRNR1          | UTR_4 & UTR_14       |
| mHBB_hHBA1              | UTR_2 & UTR_12       |
| hAlb_hHBA1              | UTR_10 & UTR_12      |
| mHBB_hHBA1-3x miR122 bs | UTR_2 & UTR_19       |
| eIF4G aptamer x1_hHBA1  | UTR_9 & UTR_12       |
| aptamer control_hHBA1   | UTR_5 & UTR_12       |
| mHBB_PNLIP-RPS3A        | UTR_2 & UTR_18       |

FIG. 8A (cont'ed)

| UTR No. (FIG. 8A) | 5' UTR (abbrev.)                                                               | SEQ ID NO. | Whole 5' UTR seq (whole 5' UTR length)                                                                                                            | SEQ ID NO. | Core 5' UTR sequence (core 5' UTR length)                                                                              |
|-------------------|--------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------|------------|------------------------------------------------------------------------------------------------------------------------|
| 2                 | mouse hemoglobin subunit beta (mHBB)                                           | 11         | AGCAUAAAAGUCACAUUU<br>GCUUCUGACAUAGUUGUG<br>UUGACUCACAACCCAGAA<br>ACAGACAUCGAAUUCGCUG<br>CCACC (79)                                               | 21         | UCACAUUUGCUUCU<br>GACAUAGUUGUGUU<br>GACUCACAACCCAG<br>AAACAGACAUC (54)                                                 |
| 7                 | Carboxypeptidase A1 (CPA1)                                                     | 12         | AGCAUAAAAGCUCGGUCCU<br>GGGAGGGUUUAAAAGCCA<br>GGGGCCGUCUCGACCUCA<br>GUCUGACCUUCCCUCCGG<br>CAGCAGCGAAUUCGCUGCC<br>ACC (97)                          | 22         | CUCGGUCCUGGGAG<br>GGUUUAAAAGCCAG<br>GGGGCCGUCUCGACC<br>UCAGUCUGACCUUCC<br>CUCCCGGCAGCAGC<br>(72)                       |
| 6                 | Defensin alpha 3 (DEFA3)                                                       | 13         | AGCAUAAAAGGGCUGCUCC<br>UUGCUAUAGAAGACCUGG<br>GACAGAGGACUGCUGUCU<br>GCCUCUCUGGUCACCCUG<br>CCUAGCUAGAGGAUCUGU<br>GACCCAGCCGAAUUCGCU<br>GCCACC (117) | 23         | GGCUGCUCCUUGC<br>UAGAAGACCUGGGAC<br>AGAGGACUGCUGUC<br>UGCCUCUCUGGUCA<br>CCCUGCCUAGCUAGA<br>GGAUCUGUGACCCCA<br>GCC (92) |
| 10                | albumin (hAlb)                                                                 | 14         | AGCAUAAAAGCUAGCUUU<br>UCUCUUCUGUCAACCCAC<br>ACGCCUUUGGCACAGAAU<br>CGCUGCCACC (66)                                                                 | 24         | CUAGCUUUUCUCUUC<br>UGUCAACCCACACG<br>CCUUUGGCACA (41)                                                                  |
| 4                 | hemoglobin subunit alpha 1 (HBA)                                               | 15         | AGCAUAAAAGACUAGUAU<br>UCUUCUGGUCCCCACAGAC<br>UCAGAGAGAACCCGAAUUC<br>GCUGCCACC (65)                                                                | 25         | ACUAGUAUUCUUCU<br>GGUCCCCACAGACUC<br>AGAGAGAACCC (40)                                                                  |
| 9                 | single copy of aptamer for translation initiation factor 4G (eIF4G aptamer x1) | 16         | AGCAUAAAAGACUCACUAU<br>UUGUUUUCGCGCCCAGUU<br>GCAAAAAGUGUCGUAU<br>GACUAAGAAUUCGCUGCCA<br>CC (76)                                                   | 26         | ACUCACUAUUUGUU<br>UUCGCGCCCAGUUGC<br>AAAAAGUGUCGUAU<br>UGACUAA (51)                                                    |

FIG. 8B

| UTR No. (FIG. 8A) | 5' UTR (abbrev.)                                      | SEQ ID NO. | Whole 5' UTR seq (whole 5' UTR length)                                                                                                                                                                   | SEQ ID NO. | Core 5' UTR sequence (core 5' UTR length)                                                                                                                                |
|-------------------|-------------------------------------------------------|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 5                 | sequence randomized - eIF4G aptamer (aptamer control) | 17         | AGCAUAAAAGGCUUCU<br>AGCAAUAGGCUAUAAU<br>UCUUCAUCGUGUUGUA<br>ACAAGCGAACUCUGAA<br>UUCGCUGCCACC (76)                                                                                                        | 27         | GCUUCUAGCAAUAGGCU<br>AUAAUUCUUCAUCGUG<br>UUGUAACAAGCGAACUC<br>U (51)                                                                                                     |
| 3                 | synthetic 5' UTR sequence (Synthetic)                 | 18         | AGCAUAAAAGAAUAA<br>GAGAGAAAAGAAGAGU<br>AAGAAGAAAUUAAGA<br>CCCCGGCGCCGAAUUCG<br>CUGCCACC (73)                                                                                                             | 28         | AAAUAAAGAGAGAAAAGA<br>AGAGUAAGAAGAAAUAU<br>AAGACCCCGGCGGCC (48)                                                                                                          |
| 1                 | Tobacco Etch Virus (TEV)                              | 19         | AGCAUAAAAGUCUCAAC<br>ACAACAUAUACAAAACA<br>AACGAAUCUCAAGCAAU<br>CAAGCAUUCUACUUCU<br>AUUGCAGCAAUUUAAA<br>UCAUUUCUUUUAAAAGC<br>AAAAGCAAUUUUCUGA<br>AAAUUUUCACCAUUUA<br>CGAACGAUAGCGAAUU<br>CGCUGCCACC (157) | 29         | UCUCAACACAACAUUA<br>CAAAACAAACGAAUCUC<br>AAGCAAUCAAGCAUUCU<br>ACUUCUAUUGCAGCAAU<br>UUAAAUCAUUUCUUUU<br>AAAGCAAAAGCAAUUUU<br>CUGAAAAUUUUCACCAU<br>UUACGAACGAUAGC<br>(132) |
| 8                 | CD8 alpha (CD8a)                                      | 20         | AGCAUAAAAGAACUUU<br>CCCCCUCGGCGCCCCA<br>CCGGCUCGCGCGGCCU<br>CCCCUCGCGCCCGAGCU<br>UCGAGCCAAGCAGCGUC<br>CUGGGGAGCGGUCGA<br>AUUCGCUGCCACC (113)                                                             | 30         | AACUUUCCCCCUCGGC<br>GCCCCACCGGCUCCCGC<br>CGCCUCCCCUCGCGCCG<br>AGCUUCGAGCCAAGCAG<br>CGUCCUGGGGAGCGCG<br>UC (88)                                                           |
|                   | common 5' end 10-nt sequence                          | 7          | AGCAUAAAAG                                                                                                                                                                                               |            |                                                                                                                                                                          |
|                   | common 3' end 15-nt sequence                          | 8          | GAAUUCGCUGCCACC                                                                                                                                                                                          |            |                                                                                                                                                                          |

FIG. 8B (cont'ed)

| UTR No. (FIG. 8A) | 3' UTR (abbrev.)                                                          | SEQ ID NO. | Whole 3' UTR seq (whole 3' UTR length)                                                                                                                           | SEQ ID NO. | Core 3' UTR sequence (core 3' UTR length)                                                                                                         |
|-------------------|---------------------------------------------------------------------------|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| 20                | Pancreatic triacylglycerol lipase (PNLIP)                                 | 31         | AGGAUCCGAGACUACUG<br>UUAUUUGACCAUGAAU<br>UGACUUCUAAUAAAAUC<br>UAGUGGUGAUGCUGUAC<br>A (69)                                                                        | 41         | GAGACUACUGUUAUU<br>UGACCAUGAAUUGA<br>CUUCUAAUAAAAUCU<br>AGUGGUGAUGC (56)                                                                          |
| 17                | ribosomal protein S3A (RPS3A)                                             | 32         | AGGAUCCAGUUCAGACU<br>UCAAAUAGUGGCCAAUA<br>AAAAGUGCUAUUUGUGA<br>UGGUUUGCUUCUGUGU<br>ACA (70)                                                                      | 42         | AGUUCAGACUCAA<br>UAGUGGCCAAUAAA<br>AGUGCUAUUUGUGA<br>UGGUUUGCUUCUG<br>(57)                                                                        |
| 21                | ribosomal protein S3A-<br>Pancreatic triacylglycerol lipase (RPS3A-PNLIP) | 33         | AGGAUCCAGUUCAGACU<br>UCAAAUAGUGGCCAAUA<br>AAAAGUGCUAUUUGUGA<br>UGGUUUGCUUCUGGAGA<br>CUACUGUUAUUUGACCA<br>AUGAAUUGACUUCUAAU<br>AAAAUCUAGUGGUGAUG<br>CUGUACA (126) | 43         | AGUUCAGACUCAA<br>UAGUGGCCAAUAAA<br>AGUGCUAUUUGUGA<br>UGGUUUGCUUCUGG<br>AGACUACUGUUAUUU<br>GACCAUGAAUUGAC<br>UUCUAAUAAAAUCU<br>GUGGUGAUGC (113)    |
| 18                | Pancreatic triacylglycerol lipase-<br>ribosomal protein S3A (PNLIP-RPS3A) | 34         | AGGAUCCGAGACUACUG<br>UUAUUUGACCAUGAAU<br>UGACUUCUAAUAAAAUC<br>UAGUGGUGAUGCAGUUC<br>AGACUUCUAAUAGUGGC<br>AAAUAAAAAGUGCUAUU<br>UGUGAUGGUUUGCUUC<br>UGUGUACA (126)  | 44         | GAGACUACUGUUAUU<br>UGACCAUGAAUUGA<br>CUUCUAAUAAAAUCU<br>AGUGGUGAUGCAGU<br>UCAGACUUCUAAUAG<br>UGGCAAUAAAAAGU<br>GCUAUUUGUGAUGG<br>UUUGCUUCUG (113) |
| 12                | hemoglobin subunit alpha 1 (hHBA1)                                        | 35         | AGGAUCCGCUGGAGCCU<br>CGGUGGCCAUGCUUCUU<br>GCCCCUUGGGCCUCCCC<br>CAGCCCCUCCUCCCCUUC<br>CUGCACCCGUACCCCGU<br>GGUCUUUGAAUAAAGUC<br>UGAGUGGGCGGCAUGUA<br>CA (124)     | 45         | GCUGGAGCCUCGGUG<br>GCCAUGCUUCUUGCC<br>CCUUGGGCCUCCCCC<br>AGCCCCUCCUCCCCUU<br>CCUGCACCCGUACCCC<br>CGUGGUCUUUGAAU<br>AAAGUCUGAGUGGGC<br>GGCA (111)  |

FIG. 8C

| UTR No. (FIG. 8A) | 3' UTR (abbrev.)                                                                       | SEQ ID NO. | Whole 3' UTR seq (whole 3' UTR length)                                                                                                                                                                                                                                                                                                                              | SEQ ID NO. | Core 3' UTR sequence (core 3' UTR length)                                                                                                                                                                                                                                                                                                                    |
|-------------------|----------------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 19                | hemoglobin subunit alpha 1 with 3 copies of miR-122 binding sites (hHBA1-3x miR122 bs) | 36         | AGGAUCCCAAACACCAUUG<br>UCACACUCCAGCUGGAGCC<br>UCGGUGGCCAUGCUUCUU<br>GCCCCUUGGGCCCCAAACAC<br>CAUUGUCACACUCCAUCCC<br>CCCAGCCCCUCCUCCCCUUC<br>CUGCACC CGUACCCCCAAA<br>CACCAUUGUCACACUCCAG<br>UGGUCUUUGAAUAAAGUC<br>UGAGUGGGCGGCAUGUAC<br>A (190)                                                                                                                       | 46         | CAAACACCAUUGUCACA<br>CUCCAGCUGGAGCCUCG<br>GUGGCCAUGCUUCUUG<br>CCCCUUGGGCCCCAAACA<br>CCAUUGUCACACUCCAU<br>CCCCCAGCCCCUCCUCC<br>CCUUCCUGCACC CGUAC<br>CCCCAAACACCAUUGU<br>CACACUCCAGUGGUCUU<br>UGAAUAAAGUCUGAGU<br>GGGCGGCA (177)                                                                                                                              |
| 16                | Carboxypeptidase A1 (CPA1)                                                             | 37         | AGGAUCCGCUGACCCUUUG<br>ACACCCUUCUUGUCCUCCU<br>CUCUGGCCCCAUCCAGGCA<br>ACCAAUAAAGUUUGAGUG<br>UACCAGGAACAGAAUCCUG<br>GGGCUUGCUGUACA (109)                                                                                                                                                                                                                              | 47         | GCUGACCCUUUGACACC<br>CUUCUUGUCCUCCUCUC<br>UGGCCCAUCCAGGCAA<br>CCAAUAAAGUUUGAGU<br>GUACCAGGAACAGAAUC<br>CUGGGGCUUGC (96)                                                                                                                                                                                                                                      |
| 15                | Defensin alpha 3 (DEFA3)                                                               | 38         | AGGAUCCGCUUGCAGAAAA<br>AGAAAAAUGAGCUAAAAU<br>UUGCUUUGAGAGCUACAG<br>GGAAUUGCUAUUACUCCUG<br>UACCUUCUGCUCAAUUCC<br>UUUCCUCAUCUCAAUAAA<br>UGCCUUGUUACAAGAUGU<br>ACA (134)                                                                                                                                                                                               | 48         | GCUUGCAGAAAAAGAAA<br>AAUGAGCUCAAAAAUUG<br>CUUUGAGAGCUACAGG<br>GAAUUGCUAUUACUCCU<br>GUACCUUCUGCUCAAUU<br>UCCUUUCCUCAUCUCAA<br>AUAAAUGCCUUGUUACA<br>AGA (121)                                                                                                                                                                                                  |
| 14                | amino-terminal enhancer of split-mitochondrially encoded 12S rRNA (AES-mtRNR1)         | 39         | AGGAUCCUGGUACUGCAU<br>GCACGCAAUGCUAGCUGCC<br>CCUUUCCCGUCCUGGGUAC<br>CCCGAGUCUCCCCGACCUC<br>GGGUCCCAGGUAUGCUCUCC<br>ACCUCCACCUGCCCCACUCA<br>CCACCUCUGCUAGUUCAG<br>ACACCUCCAAGCACGCAGC<br>AAUGCAGCUAAAACGCUU<br>AGCCUAGCCACCCCCACG<br>GGAAACAGCAGUGAUUAA<br>CUUUAGCAAUAAACGAAAG<br>UUUAACUAAGCUAUACUAA<br>CCCCAGGGUUGGUCAAUUU<br>CGUGCCAGCCACACCCUGG<br>AUGUACA (296) | 49         | CUGGUACUGCAUGCACG<br>CAAUGCUAGCUGCCCCU<br>UCCCCGUCCUGGGUACC<br>CCGAGUCUCCCCGACC<br>UCGGGUCCCAGGUAUGC<br>UCCACCUCCACCUGCC<br>CACUCACCACCUUGCU<br>AGUCCAGACACCUCCC<br>AAGCACGCAGCAAUGCA<br>GCUAAAACGCUUAGCC<br>UAGCCACACCCCCACGG<br>GAAACAGCAGUGAUUAA<br>CCUUUAGCAAUAAACGA<br>AAGUUUAAACUAAGCUAU<br>ACUAACCCCAGGGUUGG<br>UCAAUUUCGUGCCAGCC<br>ACACCCUGGA (283) |

FIG. 8C (cont'ed)

| UTR No. (FIG. 8A) | 3' UTR (abbrev.)                                    | SEQ ID NO. | Whole 3' UTR seq (whole 3' UTR length)                                                                                                                                                                                         | SEQ ID NO. | Core 3' UTR sequence (core 3' UTR length)                                                                                                                                            |
|-------------------|-----------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 11                | Xenopus beta globin (XBG)                           | 40         | AGGAUCCAGUGACU<br>GACUAGGAUCUGGU<br>UACCACUAAACCAGC<br>CUCAAGAACACCCGA<br>AUGGAGUCUCUAAG<br>CUACAUAUACCAAC<br>UUACACUUACAAAA<br>UGUUGUCCCCCAAA<br>AUGUAGCCAUUCGU<br>AUCUGCUCUAAUA<br>AAAAGAAAGUUUCU<br>UCACAUUCUGUACA<br>(171) | 50         | GGUUACCACUAAACC<br>AGCCUCAAGAACACC<br>CGAAUGGAGUCUCUA<br>AGCUACAUAUACCA<br>ACUUACACUUACAAA<br>AUGUUGUCCCCCAAA<br>AUGUAGCCAUUCGUA<br>UCUGCUCCUAAUAAA<br>AAGAAAGUUUCUUC<br>CAUUC (140) |
| 11                | Xenopus beta globin -<br>no BsrGI<br>(XBG-no BsrGI) | 51         | GGAUCCAGUGACUG<br>ACUAGGAUCUGGUU<br>ACCACUAAACCAGCC<br>UCAAGAACACCCGA<br>AUGGAGUCUCUAAG<br>CUACAUAUACCAAC<br>UUACACUUACAAAA<br>UGUUGUCCCCCAAA<br>AUGUAGCCAUUCGU<br>AUCUGCUCUAAUA<br>AAAAGAAAGUUUCU<br>UCACAUUC (164)           | 50         | GGUUACCACUAAACC<br>AGCCUCAAGAACACC<br>CGAAUGGAGUCUCUA<br>AGCUACAUAUACCA<br>ACUUACACUUACAAA<br>AUGUUGUCCCCCAAA<br>AUGUAGCCAUUCGUA<br>UCUGCUCCUAAUAAA<br>AAGAAAGUUUCUUC<br>CAUUC (140) |
|                   | common 5' end 7-nt<br>sequence                      | 9          | AGGAUCC                                                                                                                                                                                                                        |            |                                                                                                                                                                                      |
|                   | common 3' end 6-nt<br>sequence                      | 10         | UGUACA                                                                                                                                                                                                                         |            |                                                                                                                                                                                      |

FIG. 8C (cont'ed)

**CAR1 mean rank sum score of Screening in Examples 2 and 3 in freshly activated human T cells.**

| normalized to the highest rank sum in each column |                                                       |                                                   |                                                       |                                                       |                                                   |                                                       |                                                   |                                   |
|---------------------------------------------------|-------------------------------------------------------|---------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|---------------------------------------------------|-------------------------------------------------------|---------------------------------------------------|-----------------------------------|
|                                                   | R4                                                    | R4                                                | R4                                                    | R4                                                    | R4                                                | R4                                                    | R5                                                | R4-5                              |
| RM                                                | AUC CAR<br>mol/cell<br>on Total T<br>on Total T<br>R4 | CAR<br>mol/cell<br>on Total T<br>at 48 hrs<br>R 4 | Max CAR<br>mol/cell<br>on Total T<br>on Total T<br>R4 | AUC CAR<br>mol/cell<br>on Total T<br>on Total T<br>R4 | CAR<br>mol/cell<br>on Total T<br>at 48 hrs<br>R 4 | Max CAR<br>mol/cell<br>on Total T<br>on Total T<br>R4 | CAR<br>mol/cell<br>on Total T<br>at 48 hrs<br>R 5 | average<br>rank<br>Normaliz<br>ed |
| RM_61326                                          | 1.000                                                 | 0.974                                             | 1.000                                                 | 1.000                                                 | 0.893                                             | 1.000                                                 | 0.927                                             | 0.970                             |
| RM_61321                                          | 0.939                                                 | 0.969                                             | 0.935                                                 | 0.922                                                 | 1.000                                             | 0.918                                                 | 1.000                                             | 0.955                             |
| RM_61349                                          | 0.930                                                 | 0.882                                             | 0.944                                                 | 0.991                                                 | 0.987                                             | 1.000                                                 | 0.797                                             | 0.933                             |
| RM_61324                                          | 0.939                                                 | 1.000                                             | 0.913                                                 | 0.862                                                 | 0.880                                             | 0.874                                                 | 0.837                                             | 0.901                             |
| RM_61379                                          | 0.704                                                 | 0.668                                             | 0.701                                                 | 0.690                                                 | 0.742                                             | 0.636                                                 | 0.553                                             | 0.671                             |
| RM_61378                                          | 0.713                                                 | 0.646                                             | 0.727                                                 | 0.698                                                 | 0.674                                             | 0.675                                                 | 0.545                                             | 0.668                             |
| RM_61330                                          | 0.574                                                 | 0.651                                             | 0.563                                                 | 0.629                                                 | 0.592                                             | 0.693                                                 | 0.634                                             | 0.619                             |
| RM_61347                                          | 0.565                                                 | 0.603                                             | 0.554                                                 | 0.517                                                 | 0.515                                             | 0.515                                                 | 0.659                                             | 0.561                             |
| RM_61348                                          | 0.409                                                 | 0.293                                             | 0.433                                                 | 0.440                                                 | 0.438                                             | 0.468                                                 | 0.415                                             | 0.413                             |
| RM_61329                                          | 0.400                                                 | 0.445                                             | 0.372                                                 | 0.362                                                 | 0.361                                             | 0.364                                                 | 0.341                                             | 0.378                             |
| RM_61350                                          | 0.287                                                 | 0.349                                             | 0.277                                                 | 0.276                                                 | 0.236                                             | 0.286                                                 | 0.268                                             | 0.283                             |
| RM_61359                                          | 0.209                                                 | 0.223                                             | 0.216                                                 | 0.216                                                 | 0.253                                             | 0.208                                                 | 0.195                                             | 0.217                             |
| RM_61117                                          | 0.130                                                 | 0.061                                             | 0.130                                                 | 0.129                                                 | 0.129                                             | 0.130                                                 | 0.122                                             | 0.119                             |
| RM_61362                                          | 0.052                                                 | 0.122                                             | 0.052                                                 | 0.052                                                 | 0.052                                             | 0.052                                                 | 0.049                                             | 0.061                             |

**FIG. 9A**

**CAR2 mean rank sum score of Screening in Examples 2 and 3 in freshly activated human T cells.**

| RM       | normalized to the highest rank sum in each column |                                            |                                          |                                          |                                            |                                          | R5                                         | R4-5                          |
|----------|---------------------------------------------------|--------------------------------------------|------------------------------------------|------------------------------------------|--------------------------------------------|------------------------------------------|--------------------------------------------|-------------------------------|
|          | R4                                                | R4                                         | R4                                       | R4                                       | R4                                         | R4                                       |                                            |                               |
|          | AUC CAR                                           | CAR                                        | Max CAR                                  | AUC CAR                                  | CAR                                        | Max CAR                                  |                                            |                               |
|          | mol/cell<br>on Total<br>on Total<br>T R4          | mol/cell<br>on Total<br>T at 48<br>hrs R 4 | mol/cell<br>on Total<br>on Total<br>T R4 | mol/cell<br>on Total<br>on Total<br>T R4 | mol/cell<br>on Total<br>T at 48<br>hrs R 4 | mol/cell<br>on Total<br>on Total<br>T R4 | mol/cell<br>on Total<br>T at 48<br>hrs R 5 | average<br>rank<br>Normalized |
| RM_61357 | 1.000                                             | 1.000                                      | 1.000                                    | 0.948                                    | 1.000                                      | 0.948                                    | 1.000                                      | 0.985                         |
| RM_61461 | 0.978                                             | 0.961                                      | 0.996                                    | 1.000                                    | 0.978                                      | 1.000                                    | 0.958                                      | 0.982                         |
| RM_61455 | 0.900                                             | 0.900                                      | 0.899                                    | 0.900                                    | 0.895                                      | 0.883                                    | 0.915                                      | 0.899                         |
| RM_61458 | 0.891                                             | 0.853                                      | 0.894                                    | 0.874                                    | 0.690                                      | 0.883                                    | 0.829                                      | 0.845                         |
| RM_61489 | 0.873                                             | 0.680                                      | 0.903                                    | 0.887                                    | 0.847                                      | 0.887                                    | 0.815                                      | 0.842                         |
| RM_61488 | 0.803                                             | 0.693                                      | 0.815                                    | 0.779                                    | 0.753                                      | 0.792                                    | 0.739                                      | 0.768                         |
| RM_61486 | 0.725                                             | 0.673                                      | 0.733                                    | 0.792                                    | 0.891                                      | 0.788                                    | 0.716                                      | 0.760                         |
| RM_61355 | 0.777                                             | 0.857                                      | 0.762                                    | 0.680                                    | 0.642                                      | 0.684                                    | 0.770                                      | 0.739                         |
| RM_61482 | 0.677                                             | 0.647                                      | 0.689                                    | 0.710                                    | 0.631                                      | 0.710                                    | 0.570                                      | 0.662                         |
| RM_61487 | 0.659                                             | 0.519                                      | 0.698                                    | 0.589                                    | 0.766                                      | 0.541                                    | 0.622                                      | 0.628                         |
| RM_61356 | 0.633                                             | 0.844                                      | 0.590                                    | 0.407                                    | 0.528                                      | 0.403                                    | 0.723                                      | 0.590                         |
| RM_61456 | 0.563                                             | 0.703                                      | 0.559                                    | 0.528                                    | 0.496                                      | 0.550                                    | 0.549                                      | 0.564                         |
| RM_61483 | 0.441                                             | 0.429                                      | 0.465                                    | 0.654                                    | 0.751                                      | 0.636                                    | 0.387                                      | 0.538                         |
| RM_61459 | 0.568                                             | 0.494                                      | 0.597                                    | 0.545                                    | 0.437                                      | 0.563                                    | 0.420                                      | 0.518                         |
| RM_61460 | 0.493                                             | 0.617                                      | 0.474                                    | 0.511                                    | 0.345                                      | 0.558                                    | 0.484                                      | 0.497                         |
| RM_61484 | 0.441                                             | 0.437                                      | 0.449                                    | 0.511                                    | 0.659                                      | 0.485                                    | 0.430                                      | 0.487                         |
| RM_61485 | 0.336                                             | 0.316                                      | 0.341                                    | 0.385                                    | 0.437                                      | 0.390                                    | 0.207                                      | 0.345                         |
| RM_61515 | 0.354                                             | 0.307                                      | 0.363                                    | 0.338                                    | 0.397                                      | 0.338                                    | 0.289                                      | 0.341                         |
| RM_61358 | 0.288                                             | 0.372                                      | 0.280                                    | 0.273                                    | 0.299                                      | 0.268                                    | 0.263                                      | 0.292                         |
| RM_61360 | 0.306                                             | 0.294                                      | 0.311                                    | 0.286                                    | 0.251                                      | 0.290                                    | 0.286                                      | 0.289                         |
| RM_61451 | 0.223                                             | 0.221                                      | 0.225                                    | 0.221                                    | 0.236                                      | 0.221                                    | 0.136                                      | 0.212                         |
| RM_61411 | 0.183                                             | 0.182                                      | 0.185                                    | 0.182                                    | 0.183                                      | 0.182                                    | 0.131                                      | 0.176                         |
| RM_61118 | 0.131                                             | 0.143                                      | 0.132                                    | 0.104                                    | 0.127                                      | 0.104                                    | 0.070                                      | 0.116                         |
| RM_61512 | 0.070                                             | 0.065                                      | 0.070                                    | 0.078                                    | 0.071                                      | 0.078                                    | 0.028                                      | 0.066                         |

**FIG. 9B**

| RM                | MEAN_CAR<br>mol/cell on<br>Total T at<br>48hr after<br>tLNP<br>transfection | CAR<br>mol/cel<br>Standard<br>error | Nalm6<br>GFP @<br>48hr after<br>co-<br>culture | GFP<br>standard<br>error | CAR2<br>expression<br>rel. to the<br>base | CAR1<br>expression<br>rel. to the<br>base | residual<br>Nalm6<br>cells |
|-------------------|-----------------------------------------------------------------------------|-------------------------------------|------------------------------------------------|--------------------------|-------------------------------------------|-------------------------------------------|----------------------------|
| RM_61411          | 208.6                                                                       | 4.16                                | 93427                                          | 5179                     | 1.8                                       |                                           | 24%                        |
| RM_61512          | 118.5                                                                       | 0.62                                | 251144                                         | 16048                    | 1.0                                       |                                           | 66%                        |
| RM_61455          | 330.9                                                                       | 2.51                                | 93025                                          | 6690                     | 2.8                                       |                                           | 24%                        |
| RM_61456          | 280.2                                                                       | 2.96                                | 105550                                         | 6075                     | 2.4                                       |                                           | 28%                        |
| RM_61458          | 316.4                                                                       | 3.83                                | 83283                                          | 6450                     | 2.7                                       |                                           | 22%                        |
| RM_61460          | 271.1                                                                       | 2.17                                | 81816                                          | 5745                     | 2.3                                       |                                           | 21%                        |
| RM_61461          | 348.3                                                                       | 5.03                                | 45109                                          | 4827                     | 2.9                                       |                                           | 12%                        |
| RM_61487          | 291.4                                                                       | 1.92                                | 64324                                          | 3184                     | 2.5                                       |                                           | 17%                        |
| RM_61515          | 254.5                                                                       | 3.45                                | 114029                                         | 9204                     | 2.1                                       |                                           | 30%                        |
| RM_61483          | 263.5                                                                       | 1.09                                | 90047                                          | 4993                     | 2.2                                       |                                           | 24%                        |
| RM_61484          | 267.5                                                                       | 2.01                                | 100206                                         | 7097                     | 2.3                                       |                                           | 26%                        |
| RM_61485          | 242.2                                                                       | 3.45                                | 101787                                         | 5594                     | 2.0                                       |                                           | 27%                        |
| RM_61486          | 307.0                                                                       | 3.32                                | 68479                                          | 4166                     | 2.6                                       |                                           | 18%                        |
| RM_61488          | 309.1                                                                       | 2.26                                | 69423                                          | 4785                     | 2.6                                       |                                           | 18%                        |
| RM_61489          | 314.9                                                                       | 2.90                                | 87411                                          | 9658                     | 2.7                                       |                                           | 23%                        |
| RM_61451          | 210.0                                                                       | 5.02                                | 145354                                         | 6203                     | 1.8                                       |                                           | 38%                        |
| RM_61459          | 266.4                                                                       | 2.37                                | 107773                                         | 9099                     | 2.2                                       |                                           | 28%                        |
| RM_61482          | 283.8                                                                       | 5.33                                | 89588                                          | 4511                     | 2.4                                       |                                           | 23%                        |
| RM_61118          | 128.7                                                                       | 0.95                                | 267936                                         | 17639                    | 1.1                                       |                                           | 70%                        |
| RM_61362          | 105.8                                                                       | 0.61                                | 337273                                         | 33433                    |                                           | 1.0                                       | 88%                        |
| RM_61359          | 202.9                                                                       | 1.88                                | 199995                                         | 15760                    |                                           | 1.9                                       | 52%                        |
| RM_61350          | 214.1                                                                       | 3.21                                | 217705                                         | 6634                     |                                           | 2.0                                       | 57%                        |
| RM_61347          | 260.3                                                                       | 3.31                                | 116500                                         | 9291                     |                                           | 2.5                                       | 30%                        |
| RM_61348          | 235.4                                                                       | 1.25                                | 128716                                         | 7499                     |                                           | 2.2                                       | 34%                        |
| RM_61349          | 296.9                                                                       | 1.58                                | 123664                                         | 6070                     |                                           | 2.8                                       | 32%                        |
| RM_61378          | 253.5                                                                       | 1.30                                | 172136                                         | 6985                     |                                           | 2.4                                       | 45%                        |
| RM_61379          | 251.7                                                                       | 5.97                                | 145870                                         | 4822                     |                                           | 2.4                                       | 38%                        |
| RM_61321          | 315.0                                                                       | 1.58                                | 138777                                         | 6739                     |                                           | 3.0                                       | 36%                        |
| RM_61324          | 300.5                                                                       | 2.51                                | 160090                                         | 10914                    |                                           | 2.8                                       | 42%                        |
| RM_61326          | 311.0                                                                       | 0.36                                | 139901                                         | 6644                     |                                           | 2.9                                       | 37%                        |
| RM_61329          | 222.0                                                                       | 0.36                                | 199761                                         | 12157                    |                                           | 2.1                                       | 52%                        |
| RM_61330          | 258.2                                                                       | 0.63                                | 152237                                         | 6957                     | 2.2                                       |                                           | 40%                        |
| RM_61360          | 254.9                                                                       | 4.34                                | 106953                                         | 4751                     | 2.2                                       |                                           | 28%                        |
| RM_61358          | 250.9                                                                       | 3.56                                | 134392                                         | 5959                     | 2.1                                       |                                           | 35%                        |
| RM_61355          | 311.0                                                                       | 3.46                                | 97396                                          | 5124                     | 2.6                                       |                                           | 25%                        |
| RM_61356          | 303.8                                                                       | 7.12                                | 94786                                          | 6568                     | 2.6                                       |                                           | 25%                        |
| RM_61357          | 378.1                                                                       | 3.10                                | 93462                                          | 6568                     | 3.2                                       |                                           | 24%                        |
| RM_61117          | 110.6                                                                       | 0.95                                | 295687                                         | 17676                    |                                           | 1.0                                       | 77%                        |
| untransfect<br>ed | 40.9                                                                        | 0.39                                | 382317                                         | 25895                    |                                           | 0.4                                       | 100%                       |

FIG. 10

CAR expression on human CD8+ T cells at 24 hours post transfection via CD8-targeted LNP

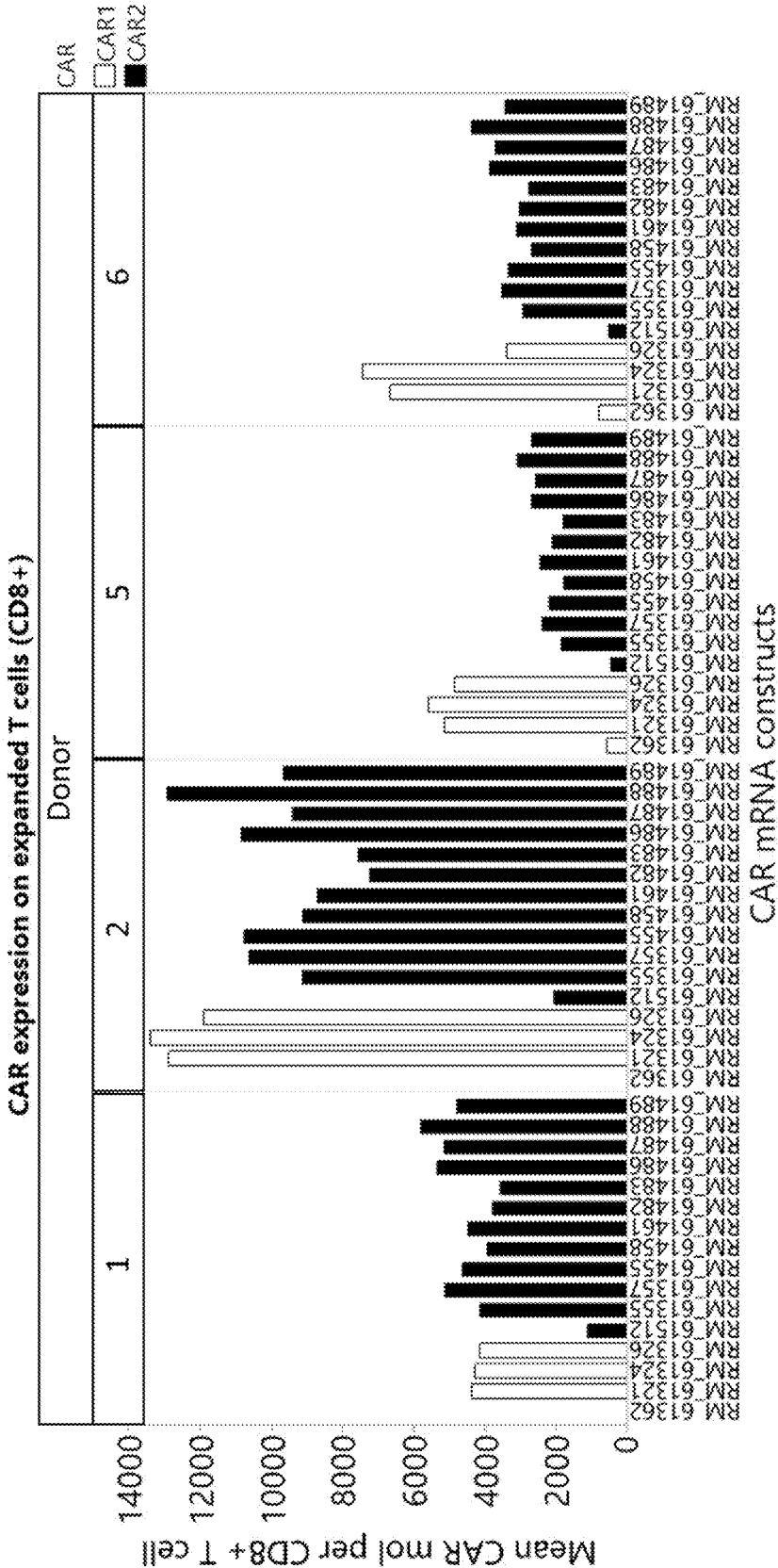


FIG. 11A

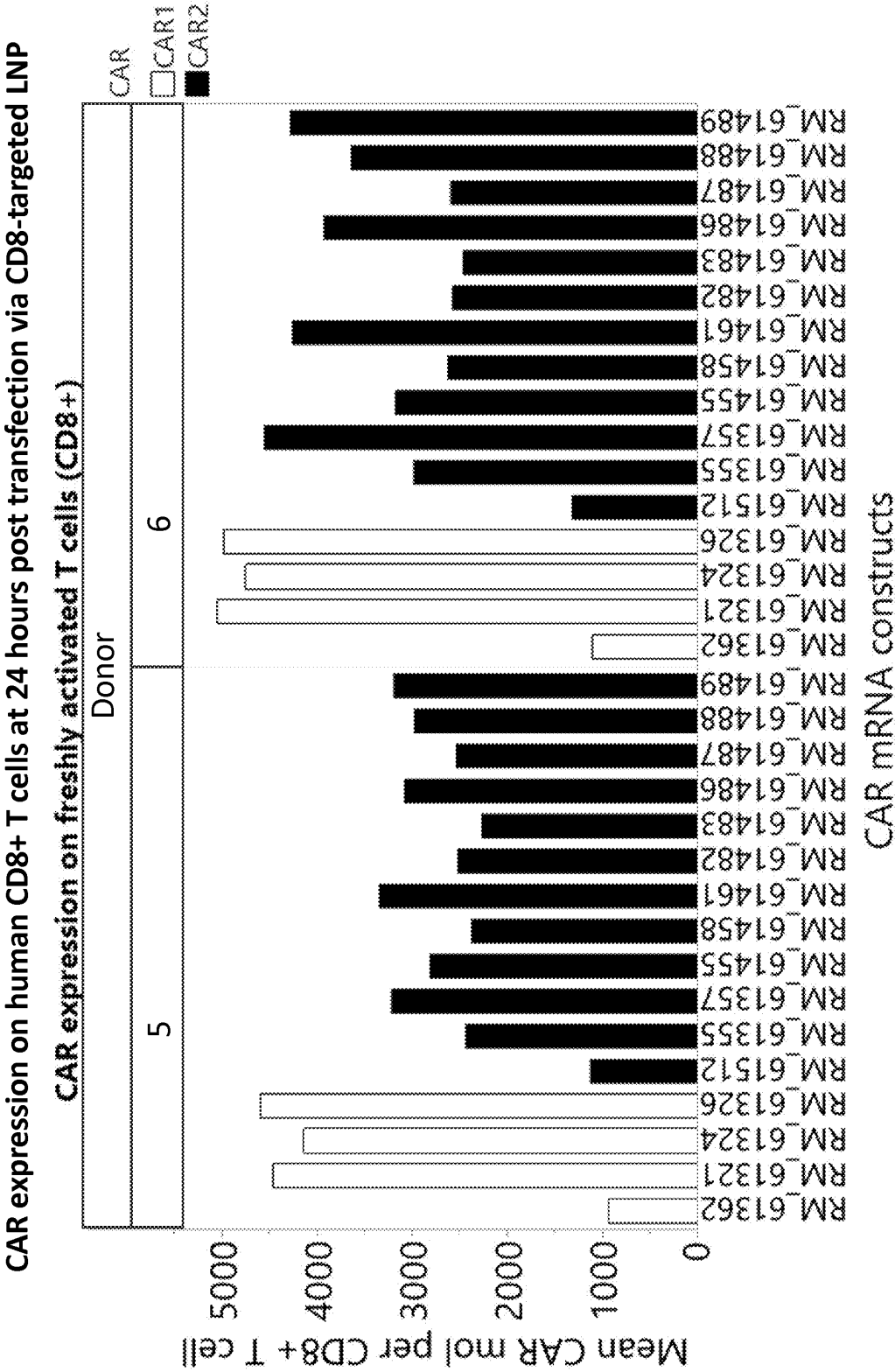


FIG. 11B

CAR expression on human CD8+ T cells at 24 hours post transfection via CD8-targeted LNP

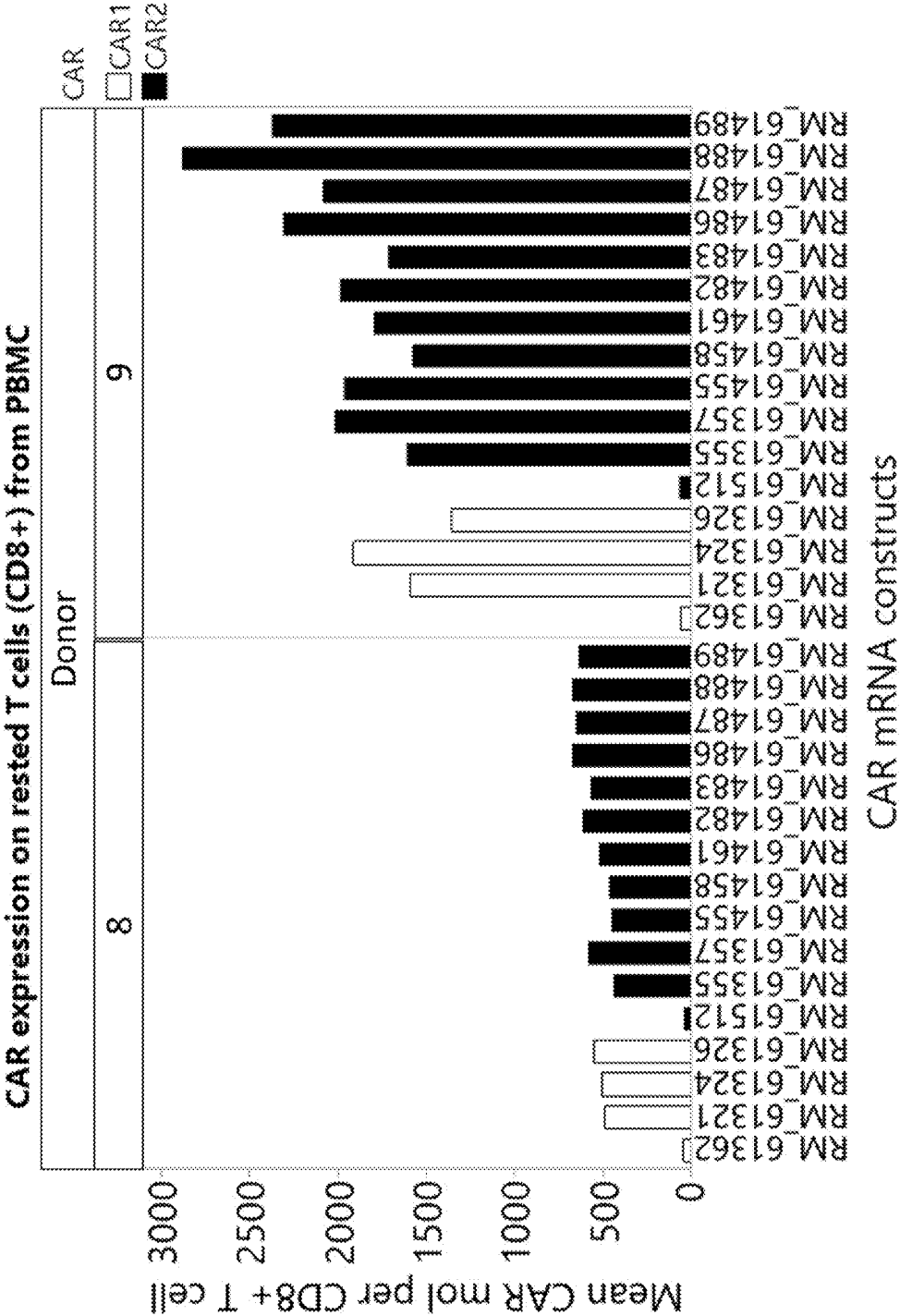


FIG. 11C

10  $\mu$ g dose

Pan T cells

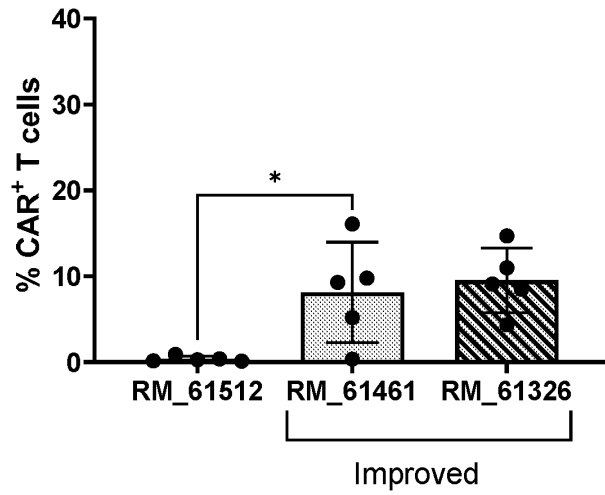
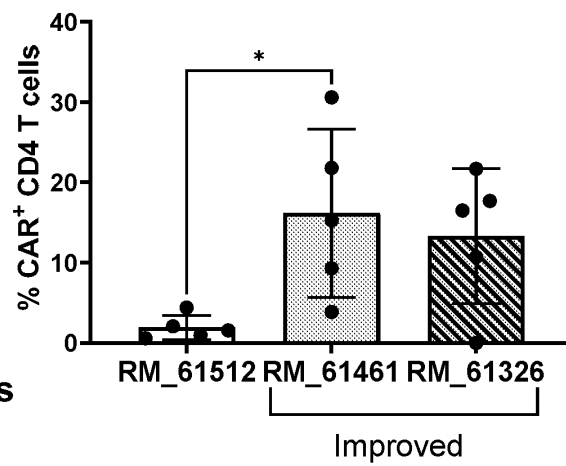
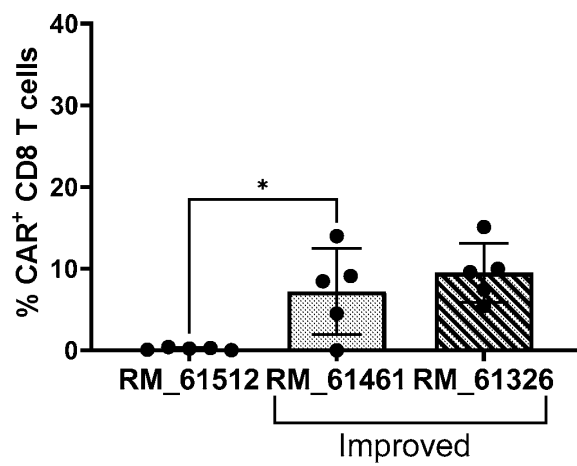
CD4<sup>+</sup> T cellsCD8<sup>+</sup> T cells

FIG. 12

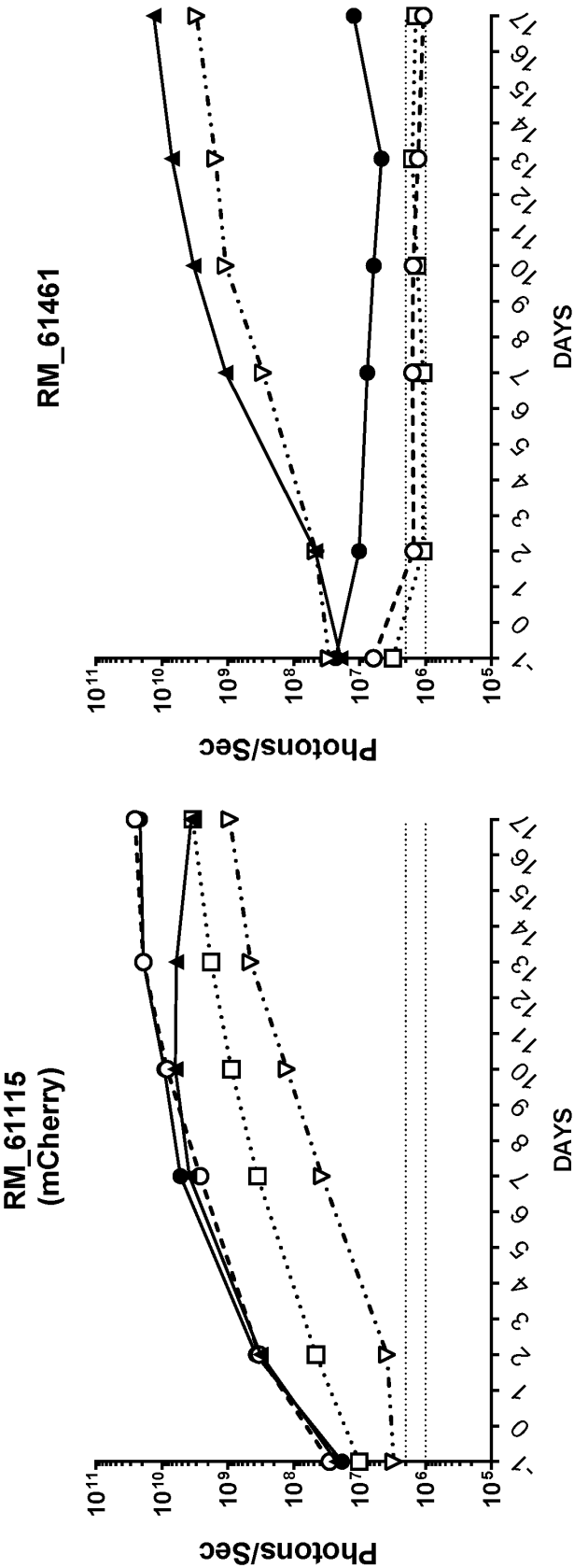


FIG. 13

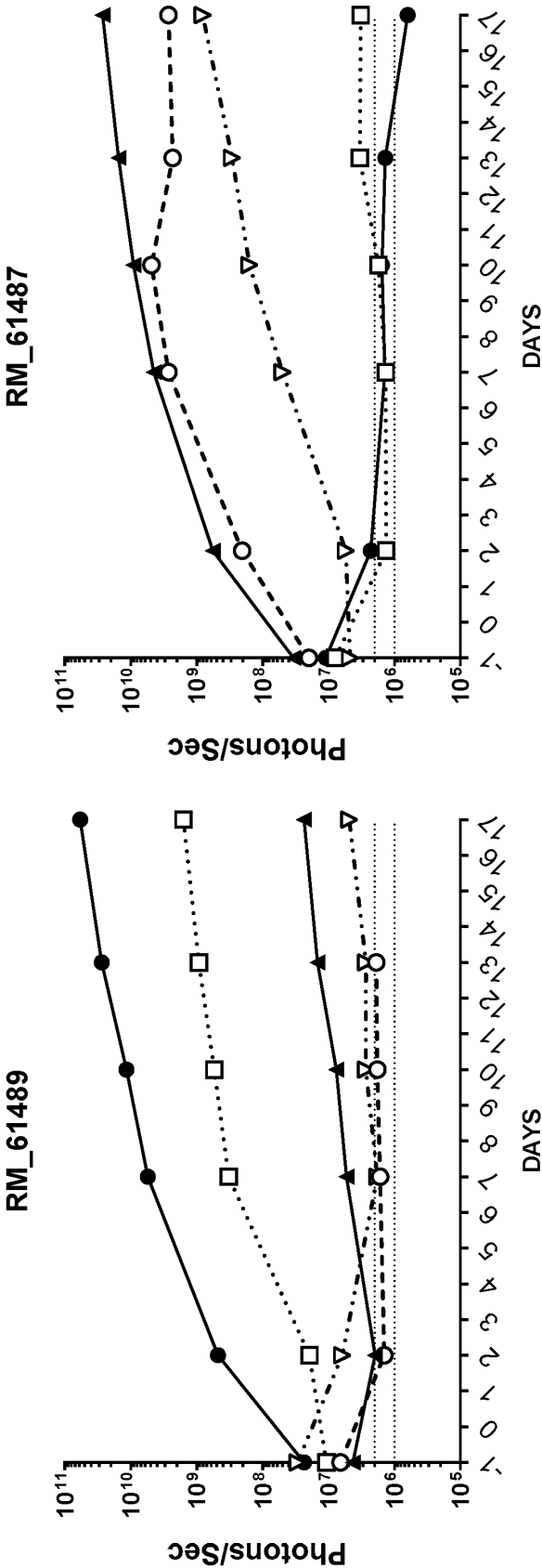


FIG. 13 (cont'ed)

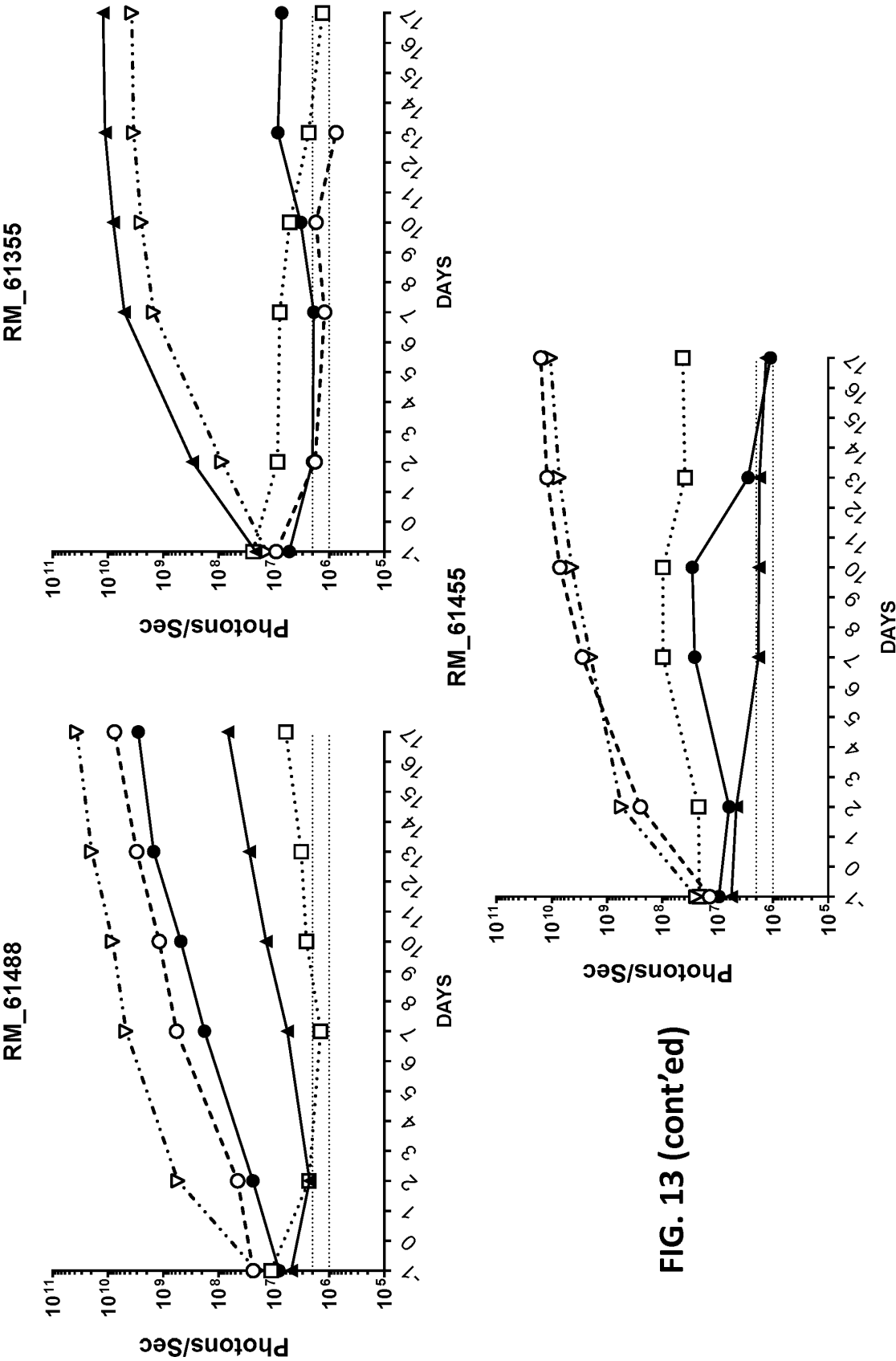


FIG. 13 (cont'ed)

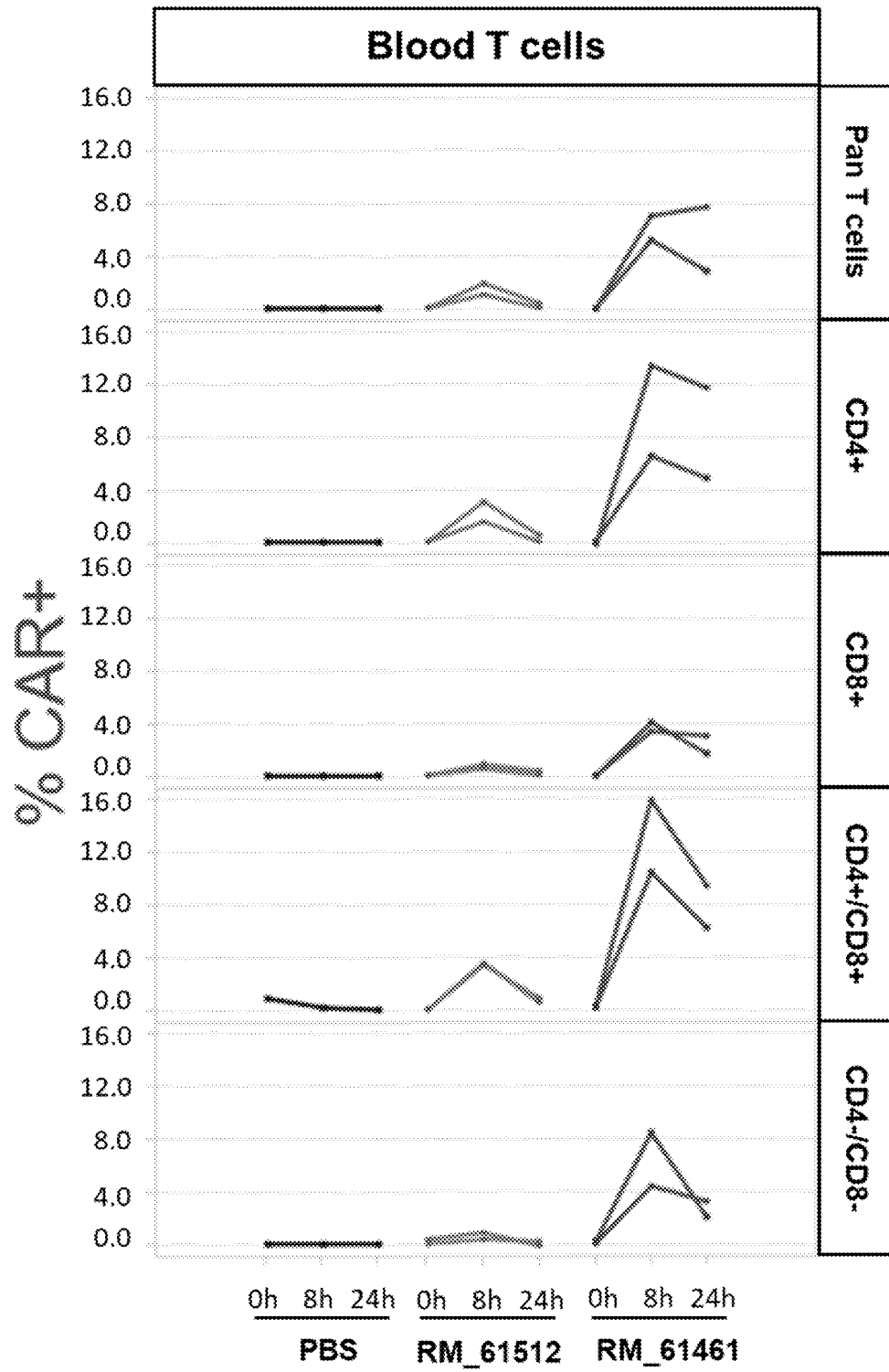
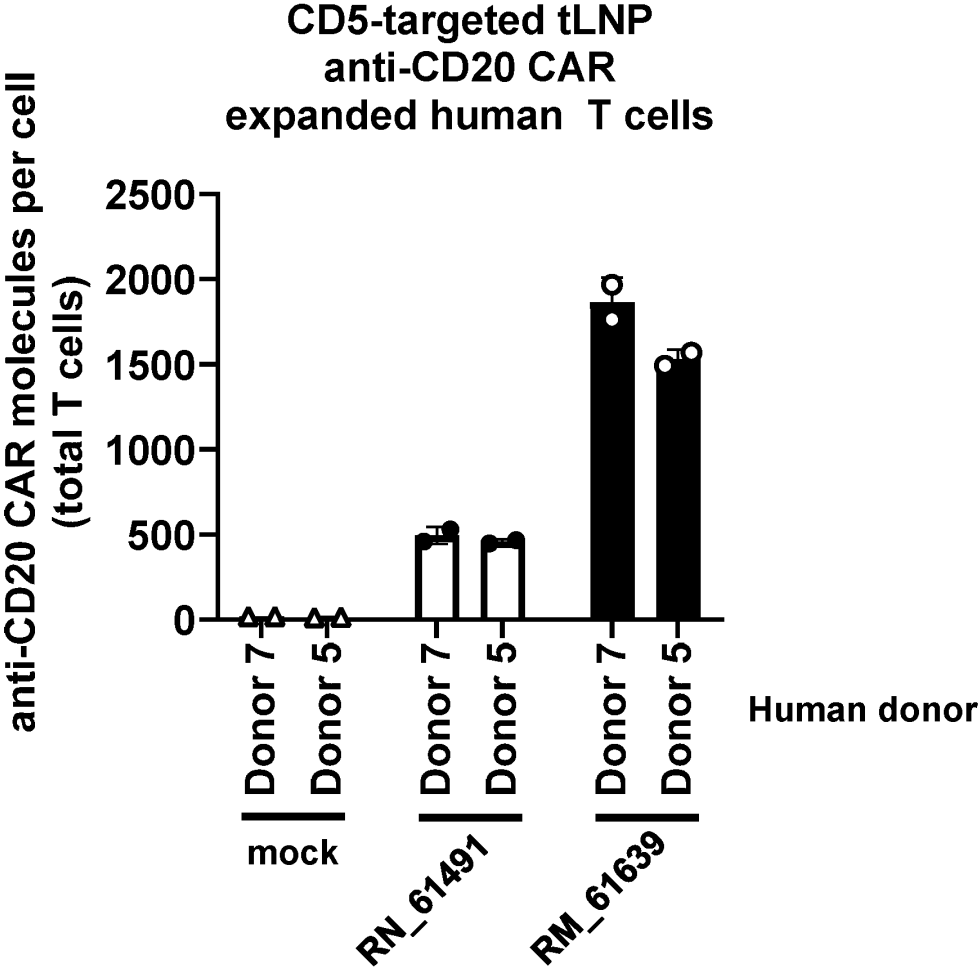


FIG. 14



| SEQ ID NO | RM       | 5' UTR | ORF encoded CAR-Codon optimization method (OPT) | 3' UTR      |
|-----------|----------|--------|-------------------------------------------------|-------------|
| 177       | RM_61491 | TEV    | CAR25-OPT6                                      | XBG         |
| 178       | RM_61639 | mHBB   | CAR25-OPT4                                      | PNLIP-RPS3A |

FIG. 15

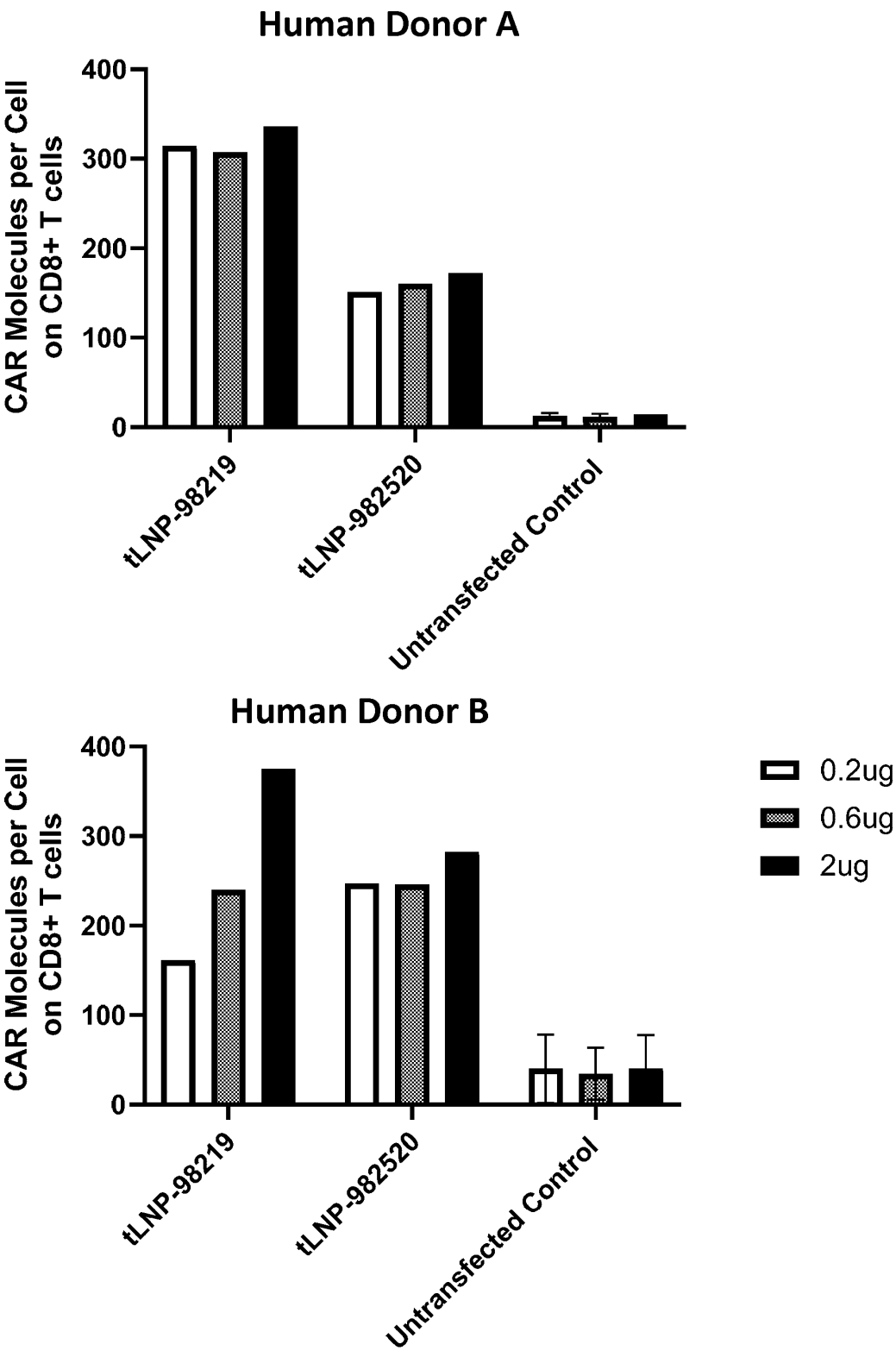


FIG. 16A

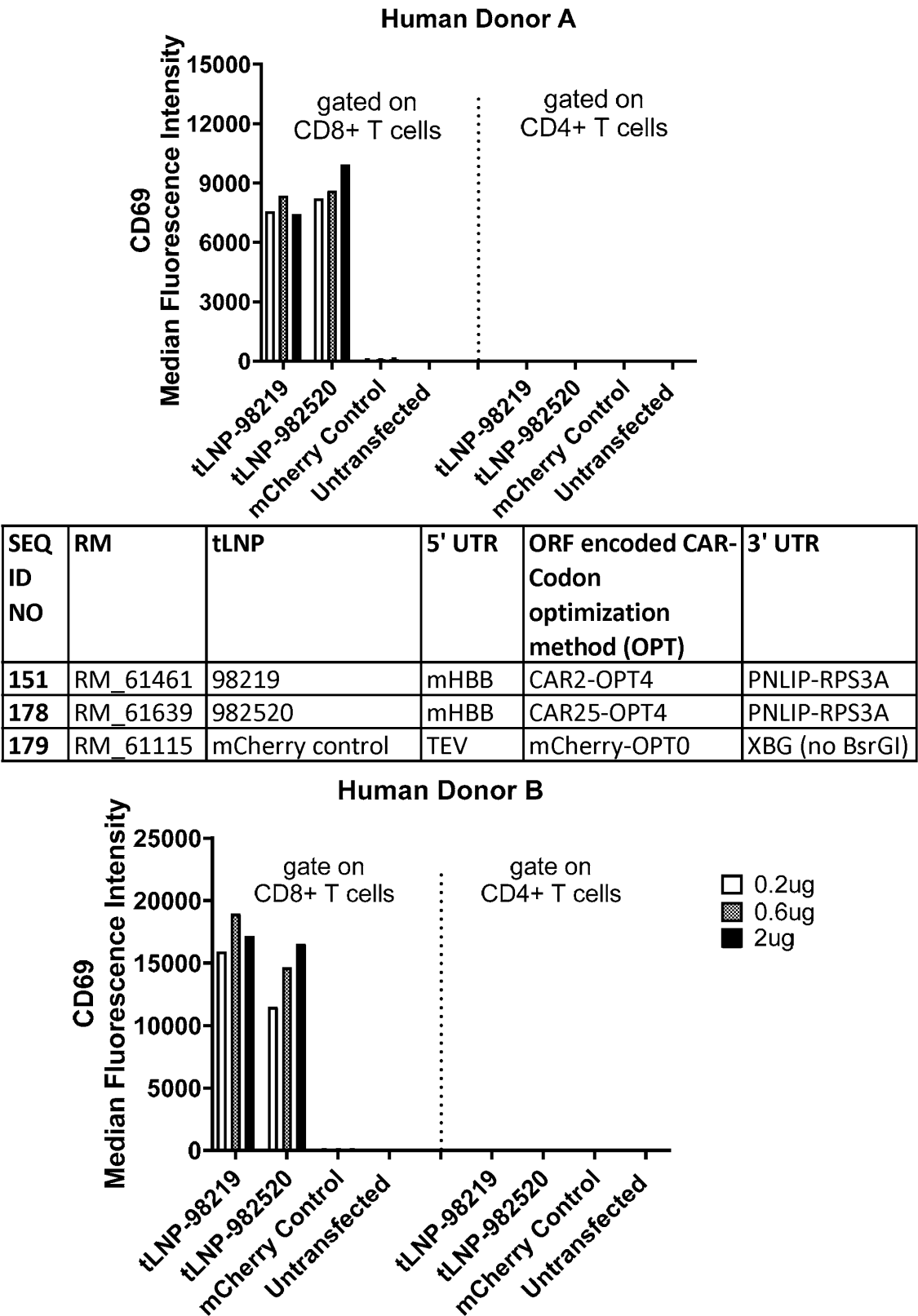
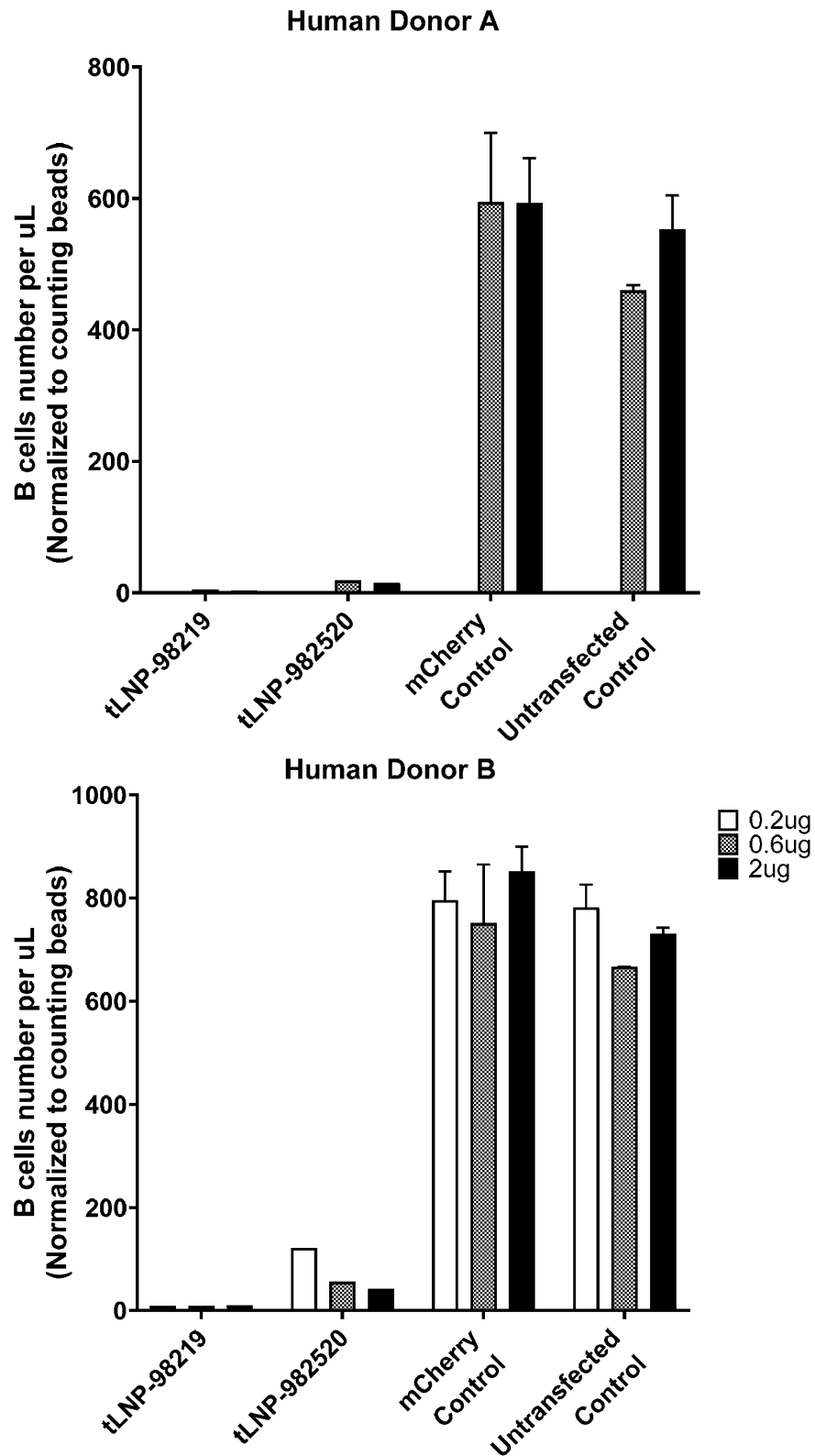
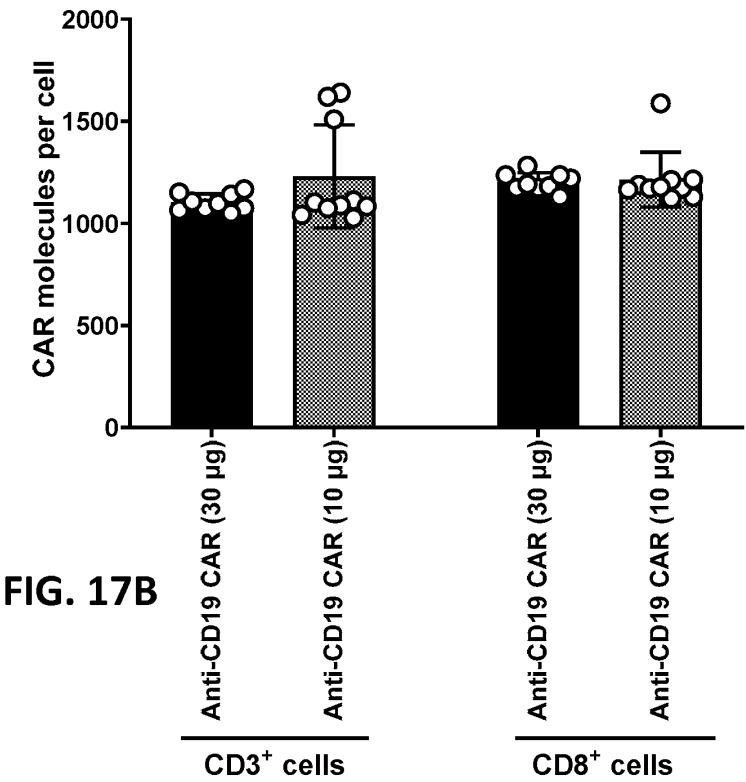
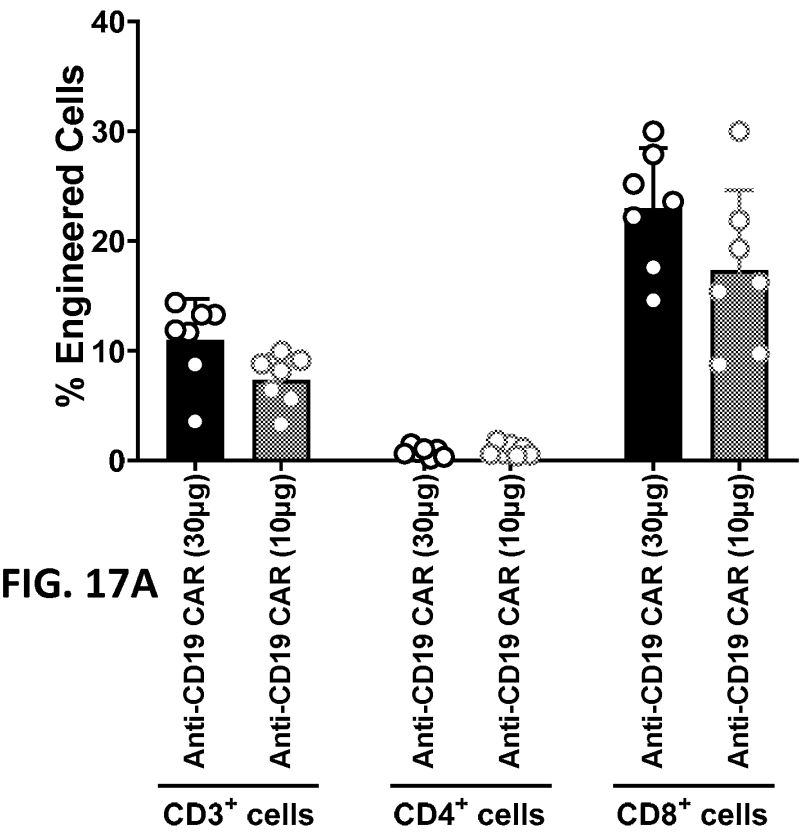
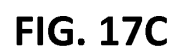


FIG. 16B

**FIG. 16C**





**FIG. 17C**

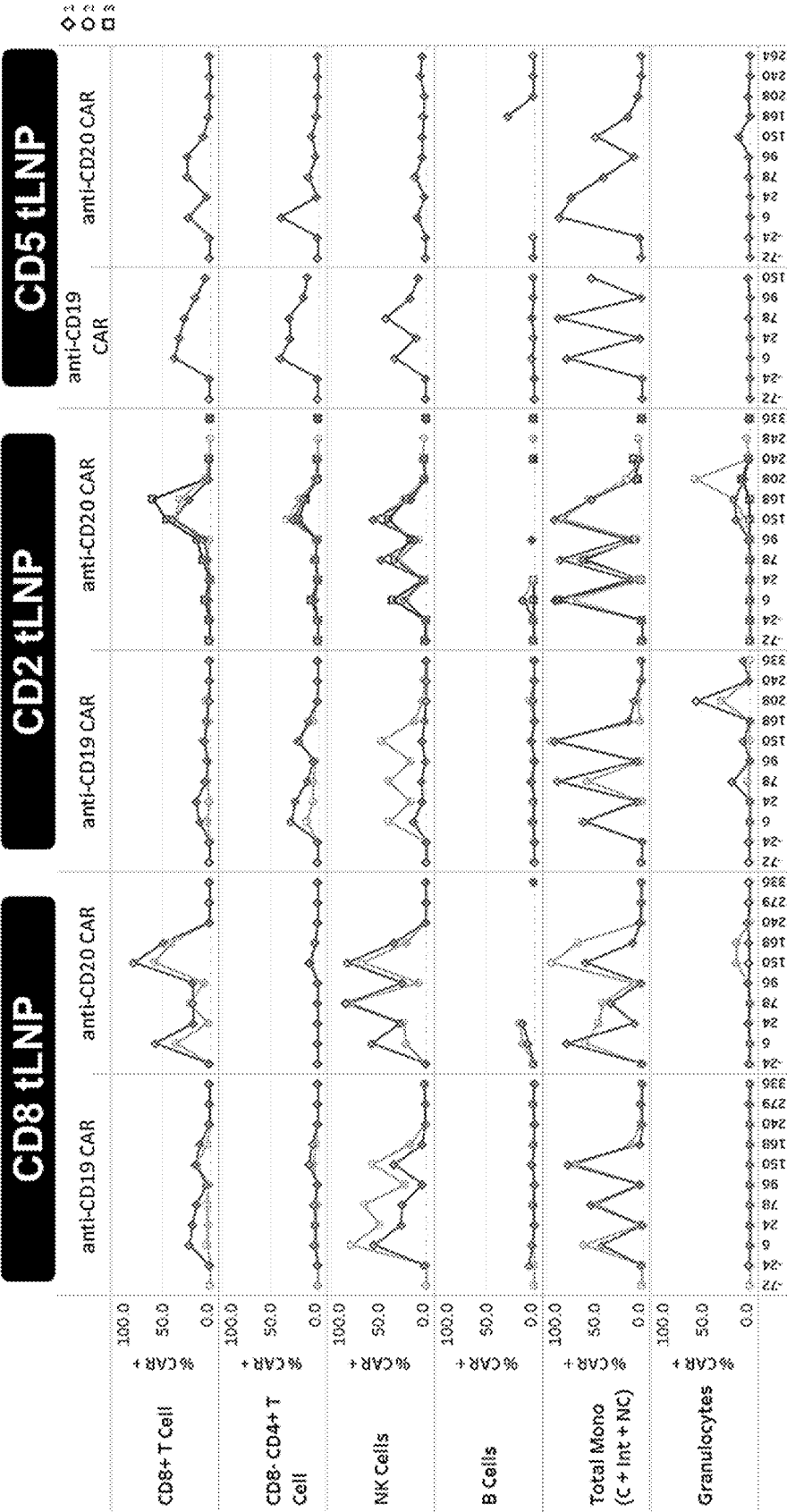


FIG. 18A

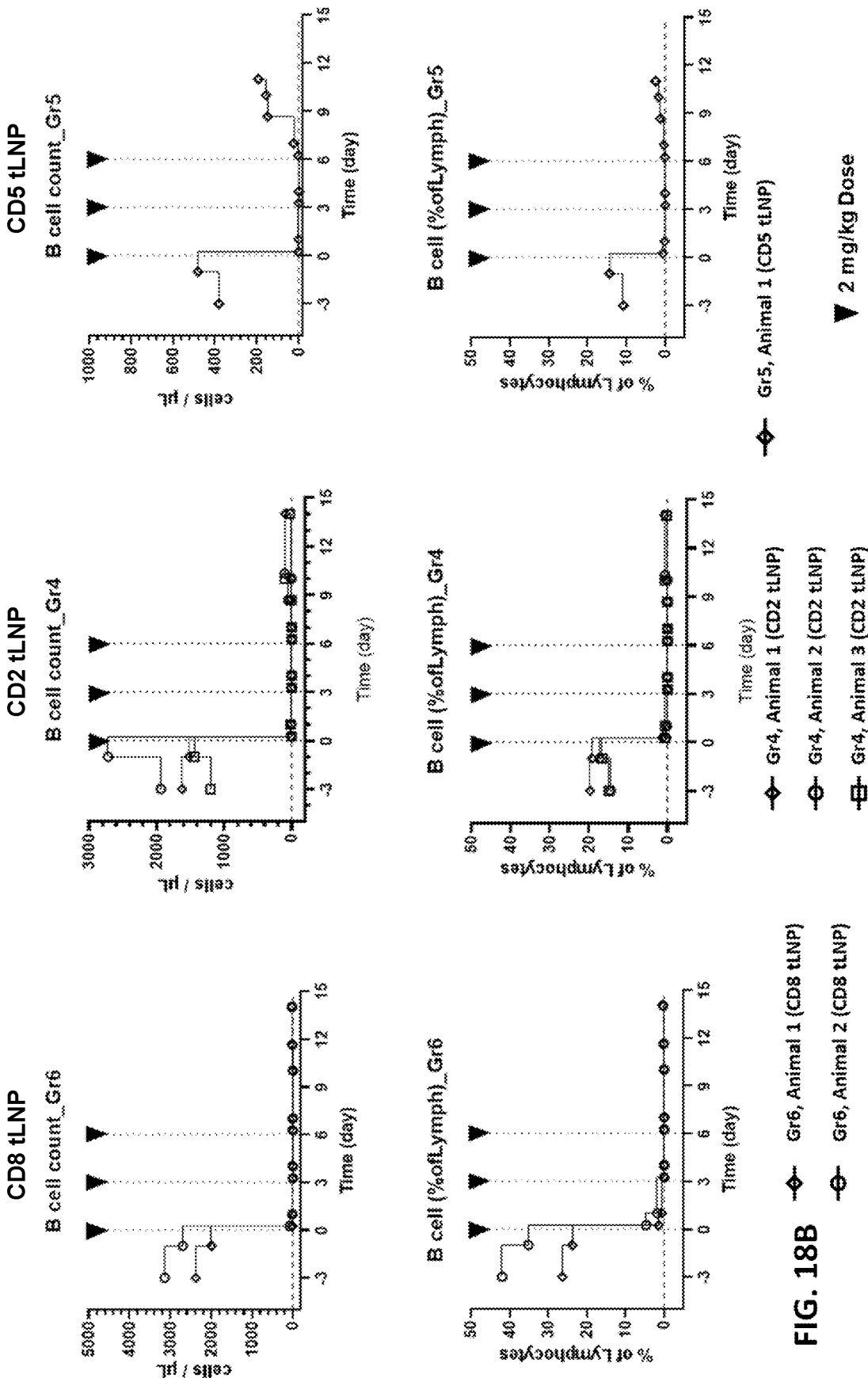


FIG. 18B

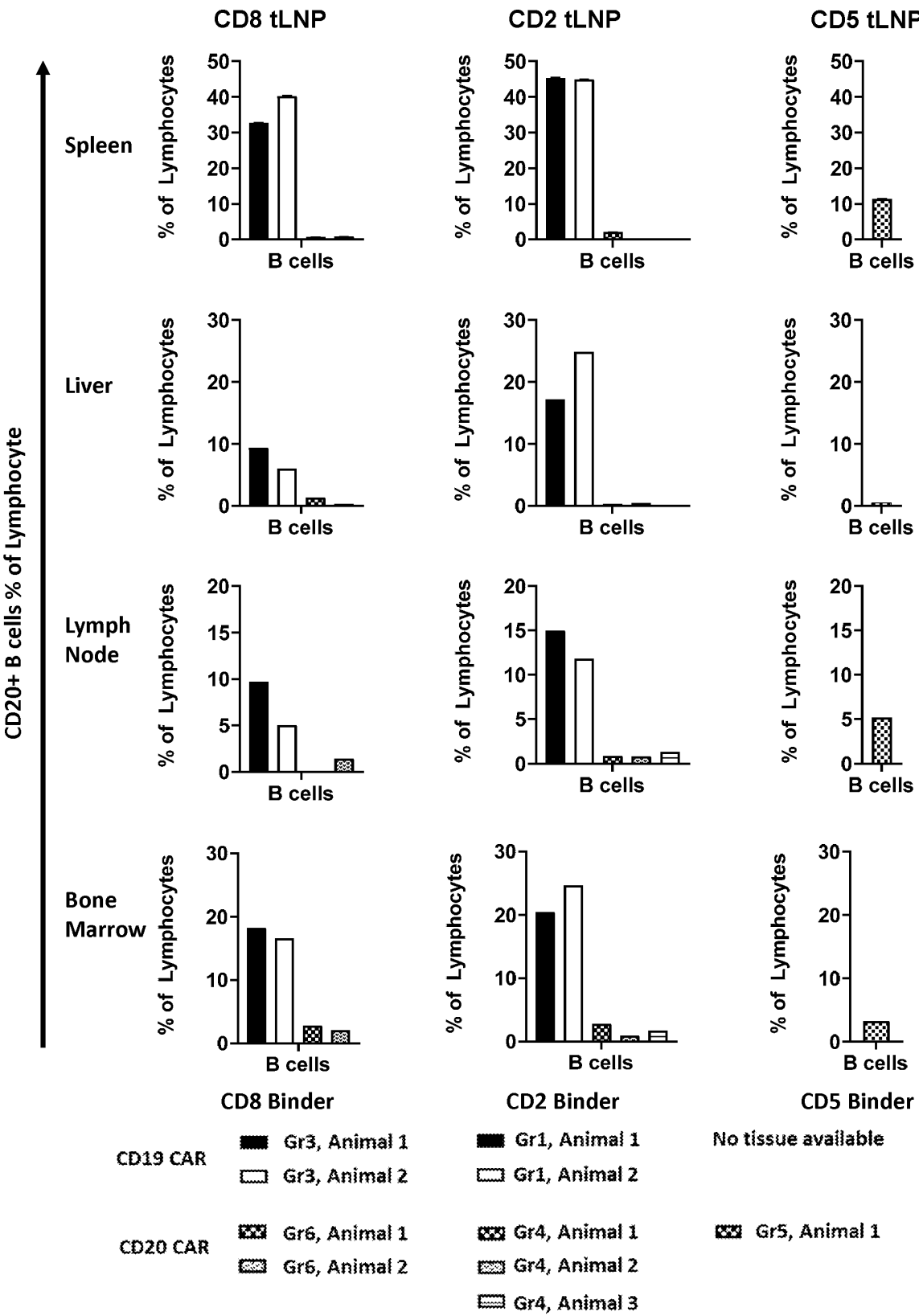


FIG. 18C

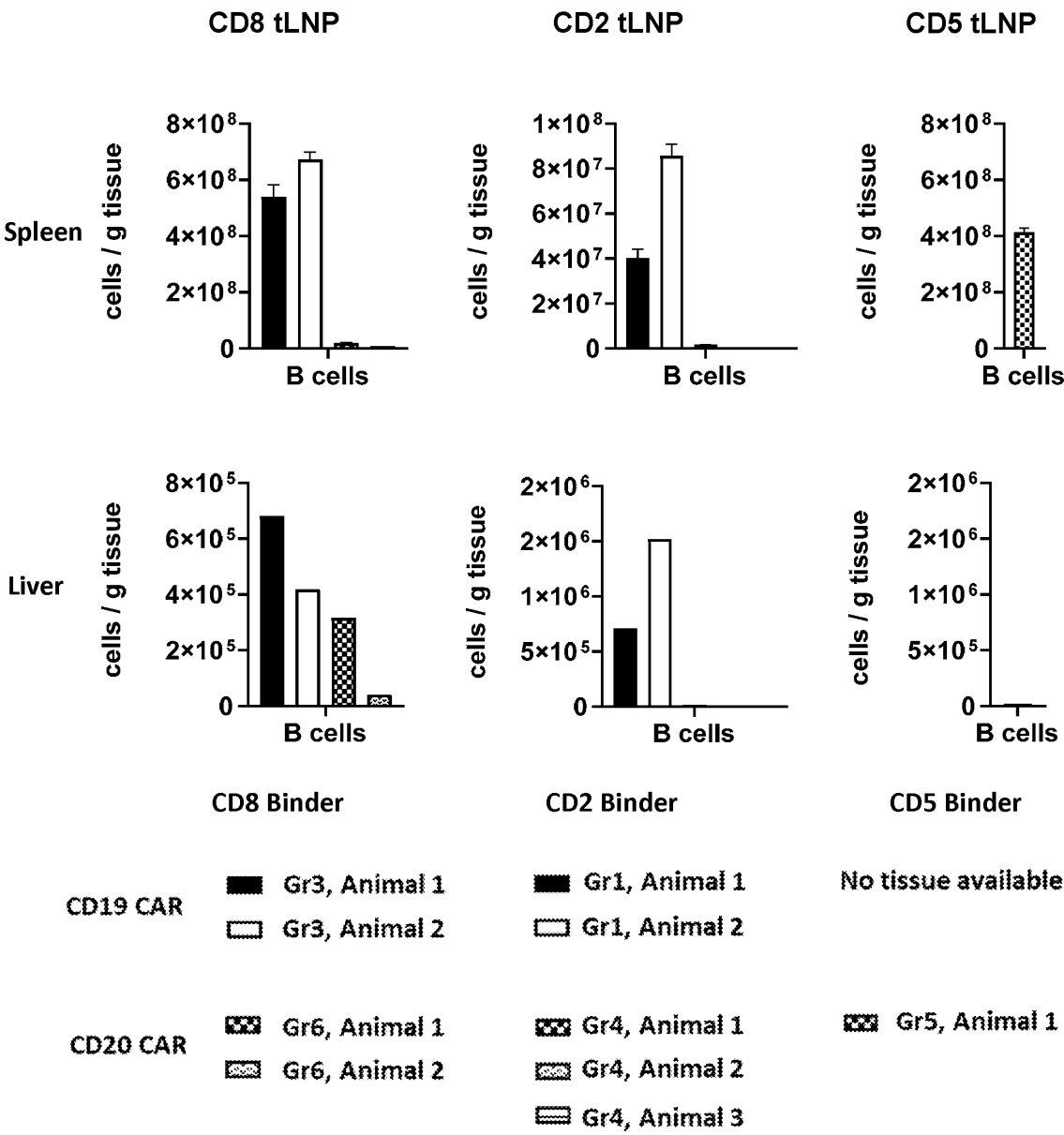
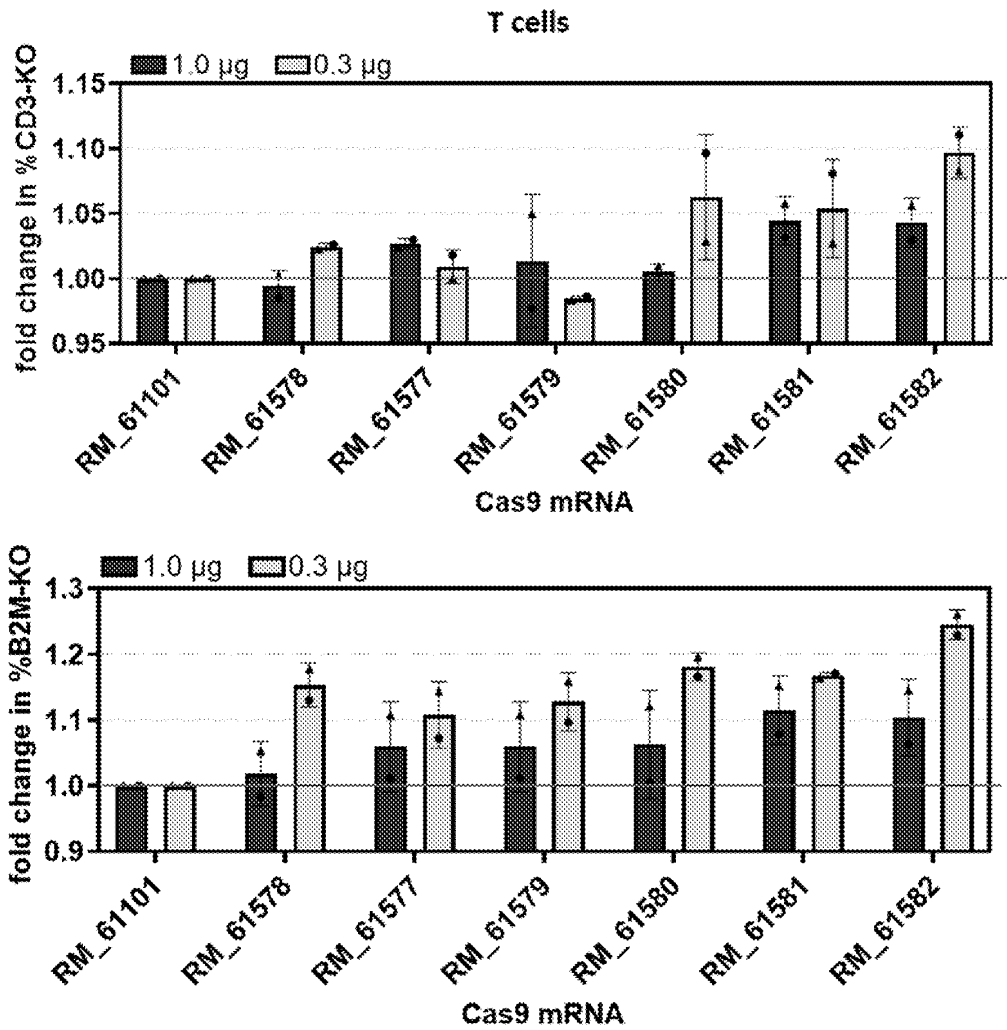


FIG. 18D



| RM       | SEQ ID NO | 5' UTR | ORF encoded Cas9-Codon optimization method (OPT) | 3' UTR      |
|----------|-----------|--------|--------------------------------------------------|-------------|
| RM_61101 | 306       | TEV    | Cas9-OPT8                                        | XBG         |
| RM_61577 | 307       | mHBB   | Cas9'-OPT8                                       | PNLIP-RPS3A |
| RM_61578 | 308       | mHBB   | Cas9'-OPT8                                       | hHBA1       |
| RM_61579 | 309       | mHBB   | Cas9-OPT2                                        | PNLIP-RPS3A |
| RM_61580 | 310       | mHBB   | Cas9-OPT2                                        | hHBA1       |
| RM_61581 | 311       | mHBB   | Cas9-OPT4                                        | PNLIP-RPS3A |
| RM_61582 | 312       | mHBB   | Cas9-OPT4                                        | hHBA1       |

Cas9': Cas9 with two silent mutations to remove BspQI restriction sites.

**FIG. 19A**

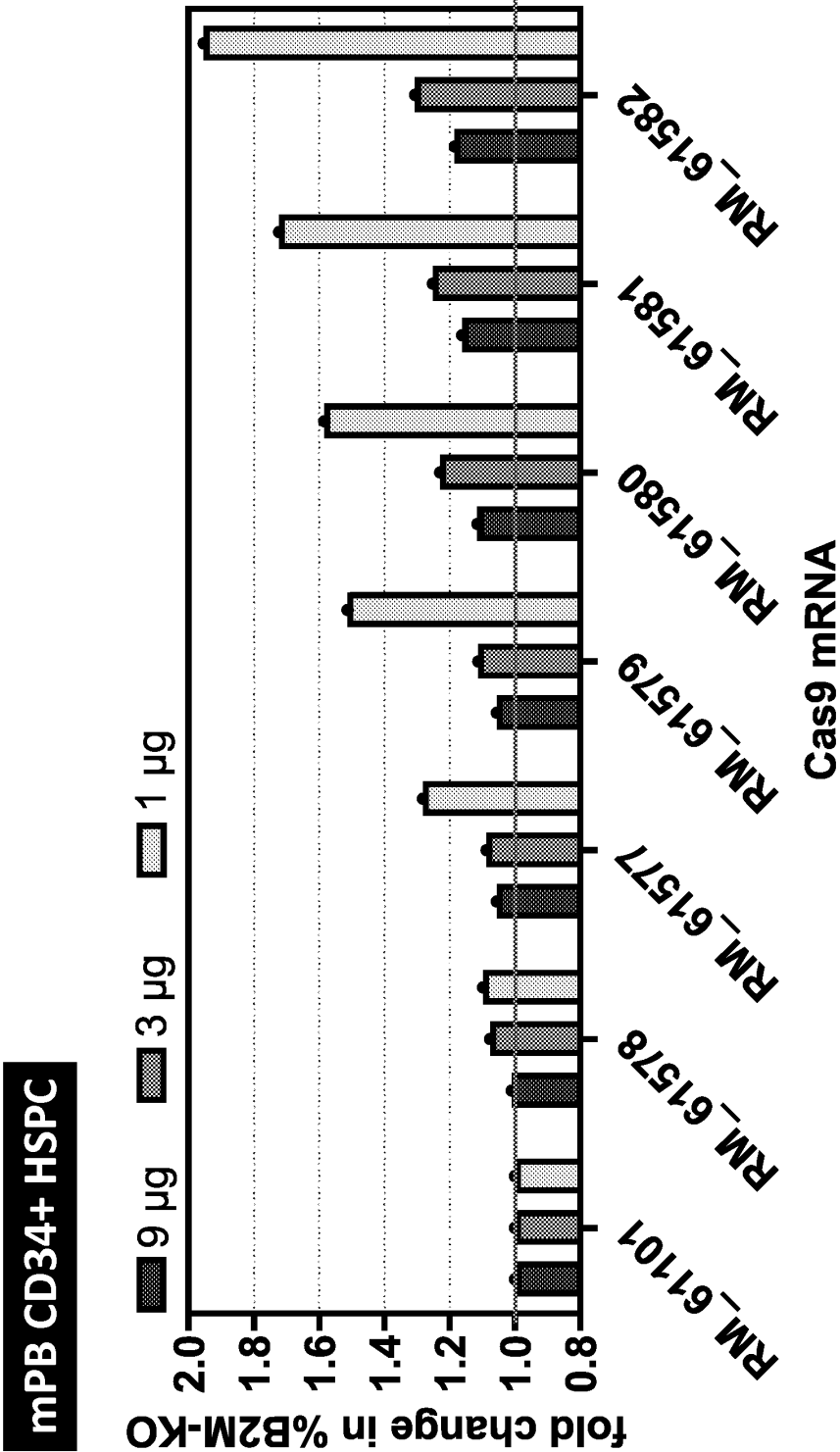
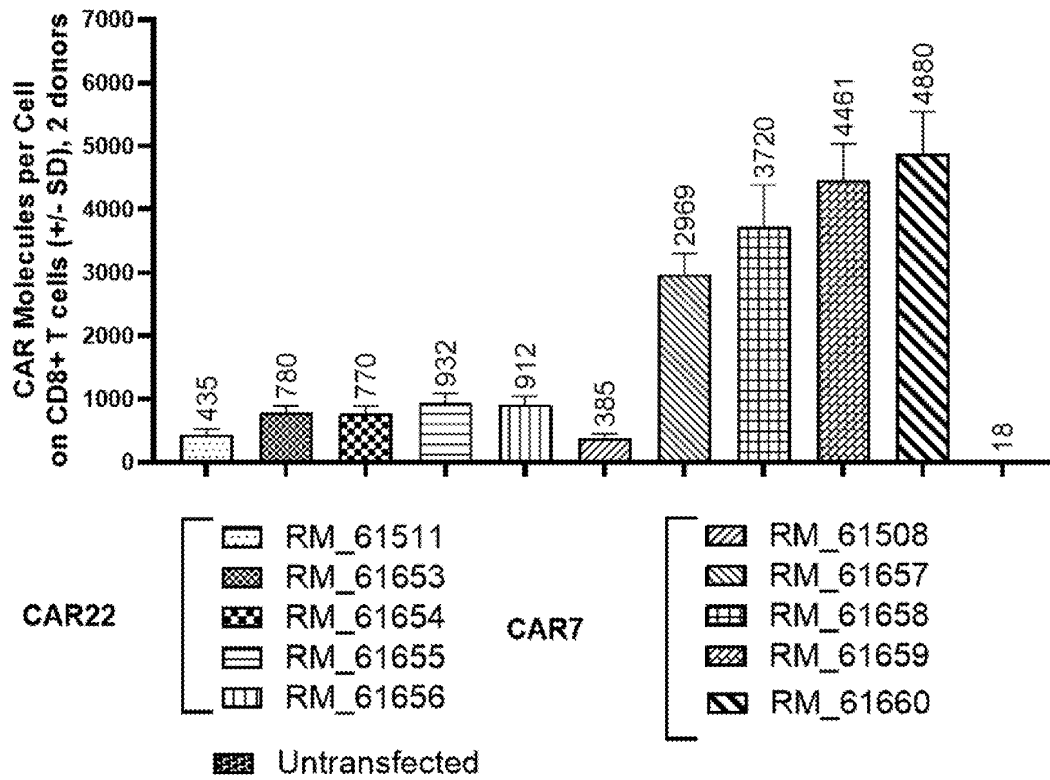


FIG. 19B



| SEQ ID NO | RM       | 5' UTR | ORF encoded CAR-Codon optimization method (OPT) | 3' UTR                |
|-----------|----------|--------|-------------------------------------------------|-----------------------|
| 315       | RM_61511 | TEV    | CAR22-OPT8                                      | XBG                   |
| 316       | RM_61653 | mHBB   | CAR22-OPT4                                      | PNLIP-RPS3A           |
| 317       | RM_61654 | mHBB   | CAR22-OPT4                                      | hHBA1-3x<br>miR122 bs |
| 318       | RM_61655 | mHBB   | CAR22-OPT0                                      | PNLIP-RPS3A           |
| 319       | RM_61656 | mHBB   | CAR22-OPT0                                      | hHBA1-3x<br>miR122 bs |
| 320       | RM_61508 | TEV    | CAR7-OPT6                                       | XBG                   |
| 321       | RM_61657 | mHBB   | CAR7-OPT4                                       | PNLIP-RPS3A           |
| 322       | RM_61658 | mHBB   | CAR7-OPT4                                       | hHBA1-3x<br>miR122 bs |
| 323       | RM_61659 | mHBB   | CAR7-OPT0                                       | PNLIP-RPS3A           |
| 324       | RM_61660 | mHBB   | CAR7-OPT0                                       | hHBA1-3x<br>miR122 bs |

FIG. 20

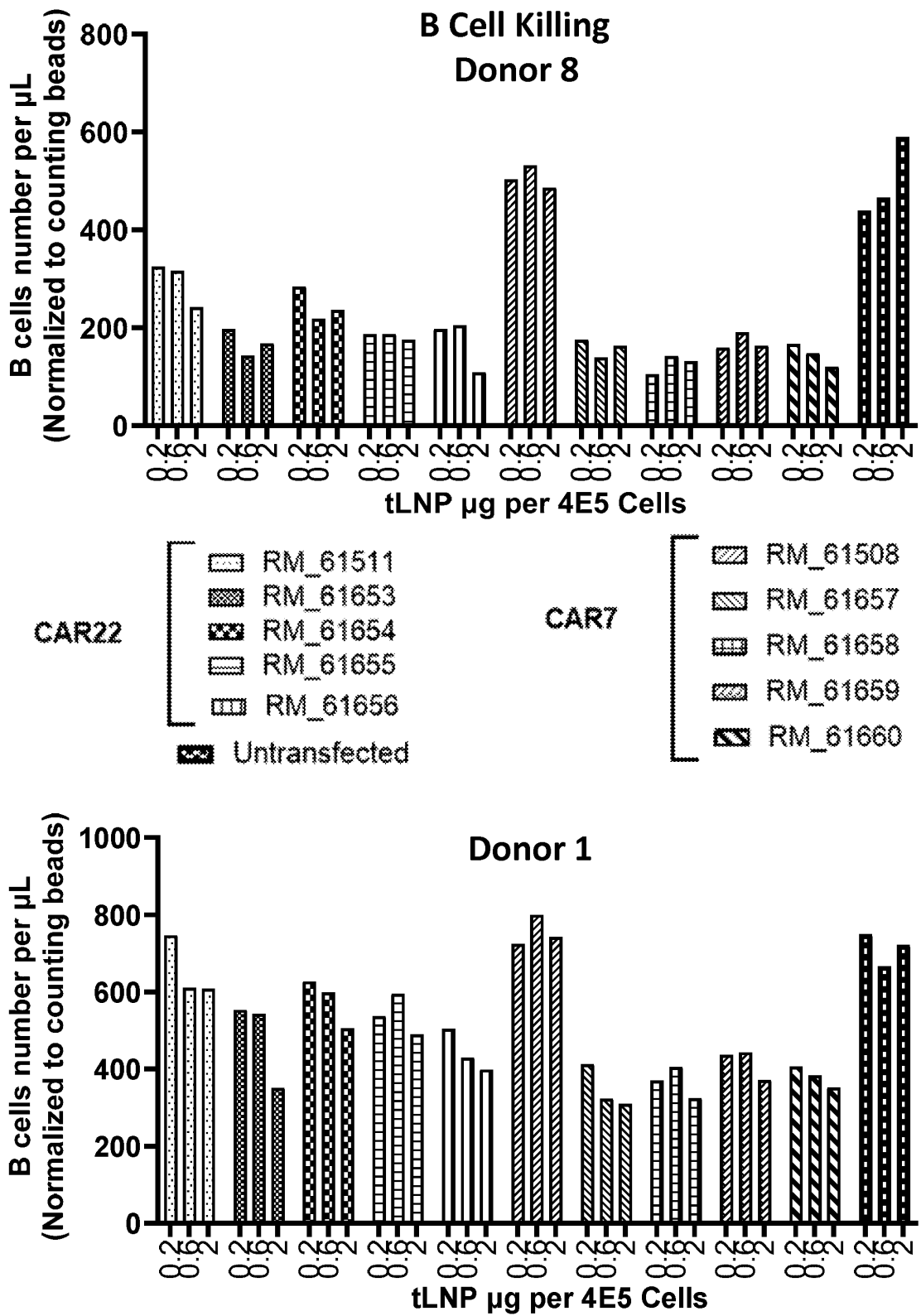


FIG. 21A

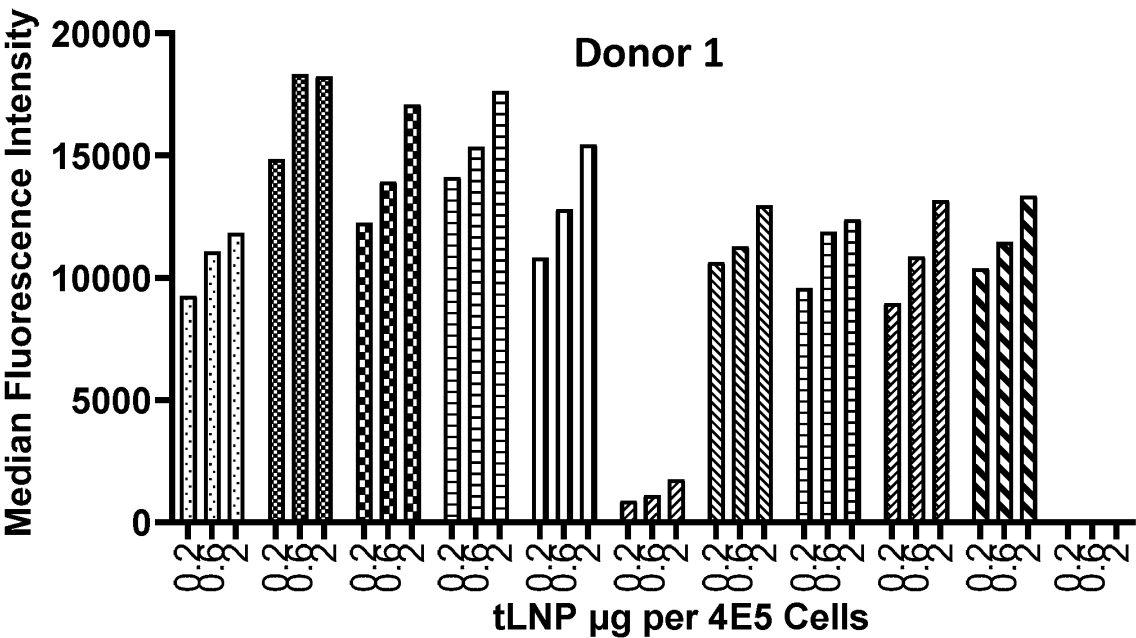
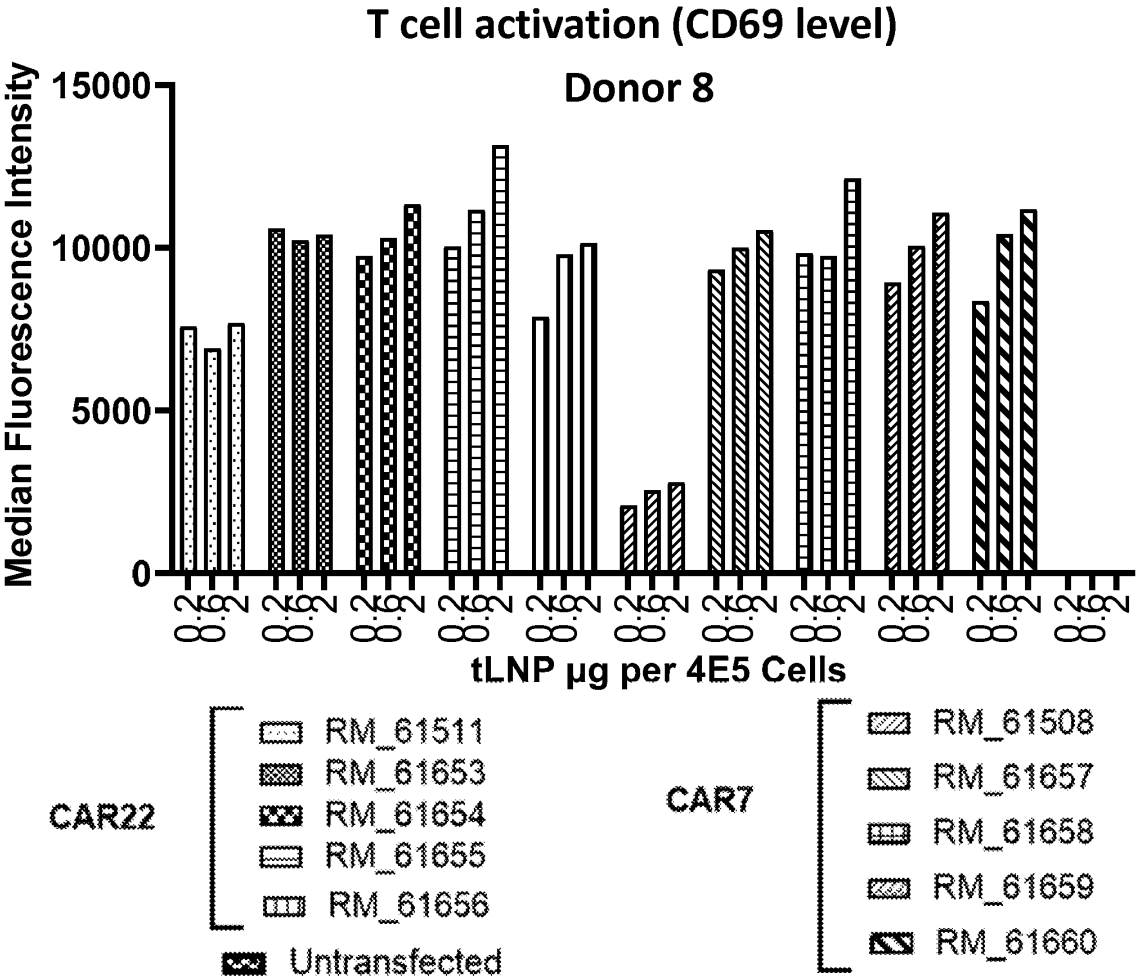
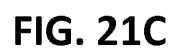


FIG. 21B



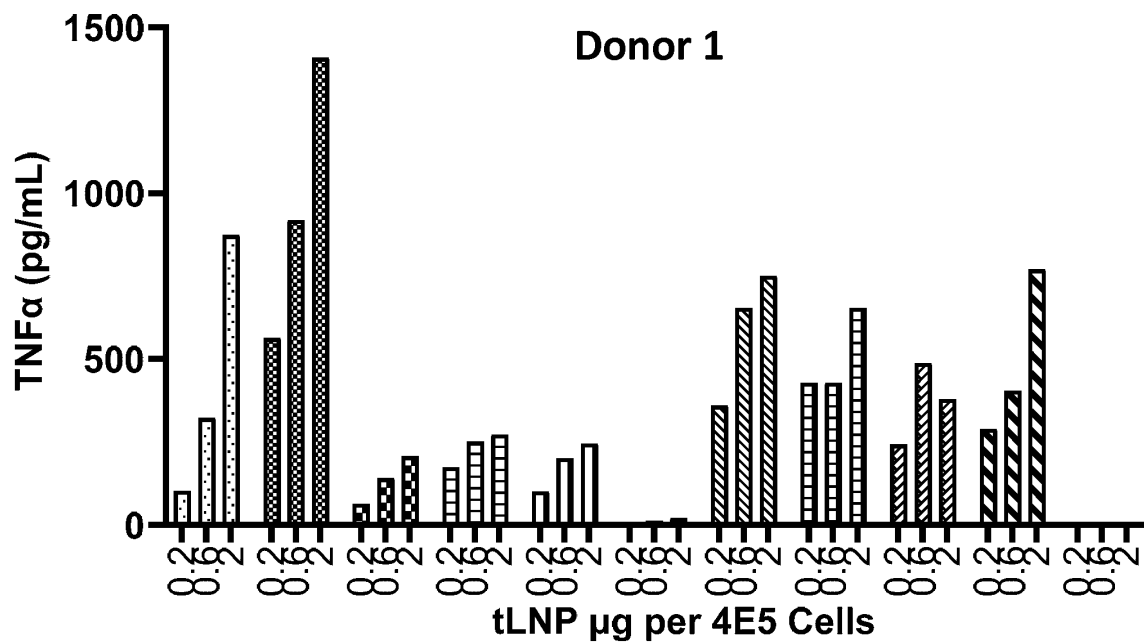
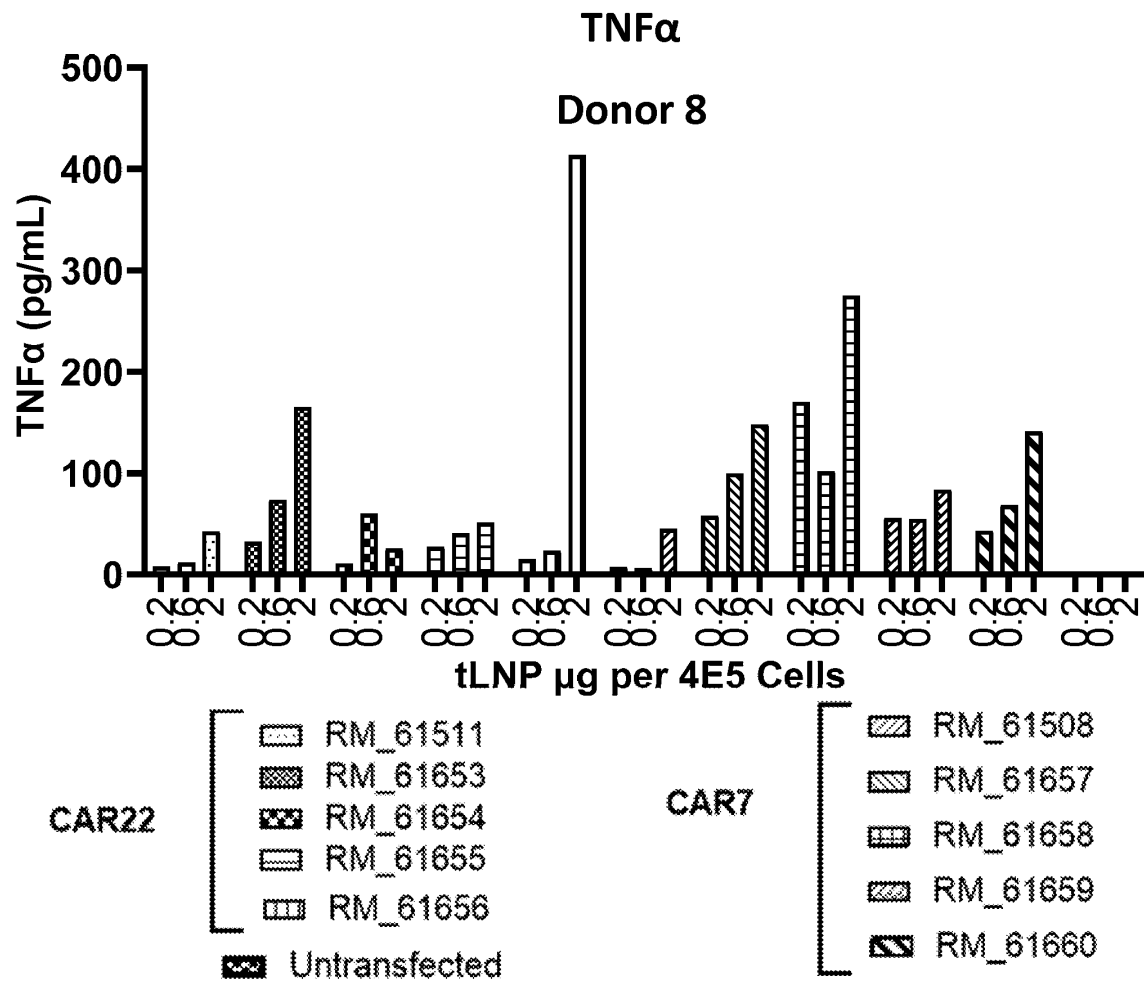


FIG. 21D

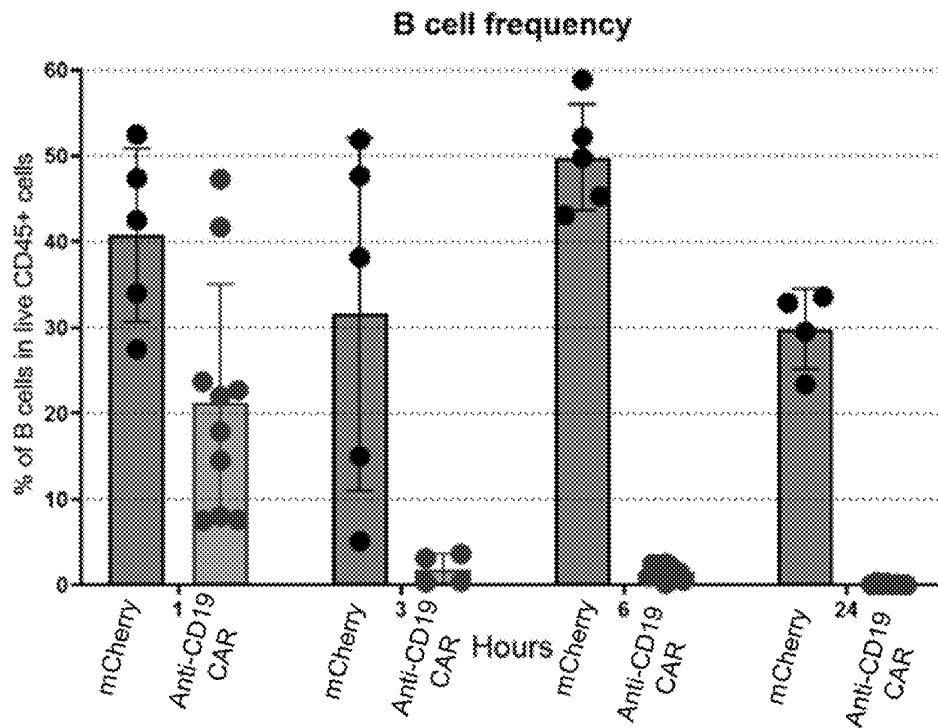


FIG. 22A

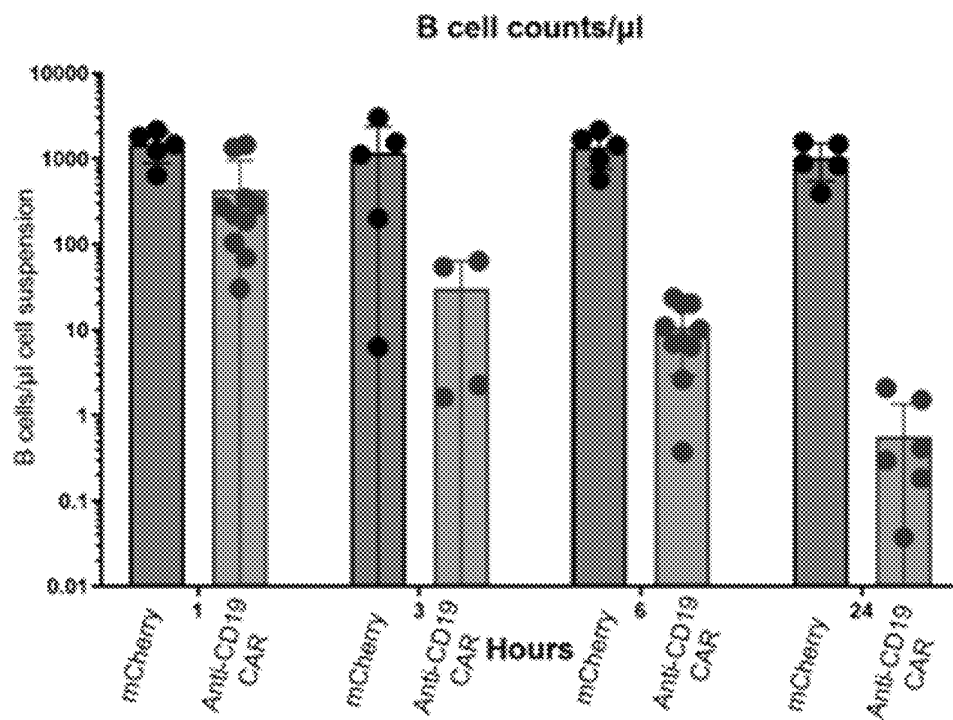


FIG. 22B

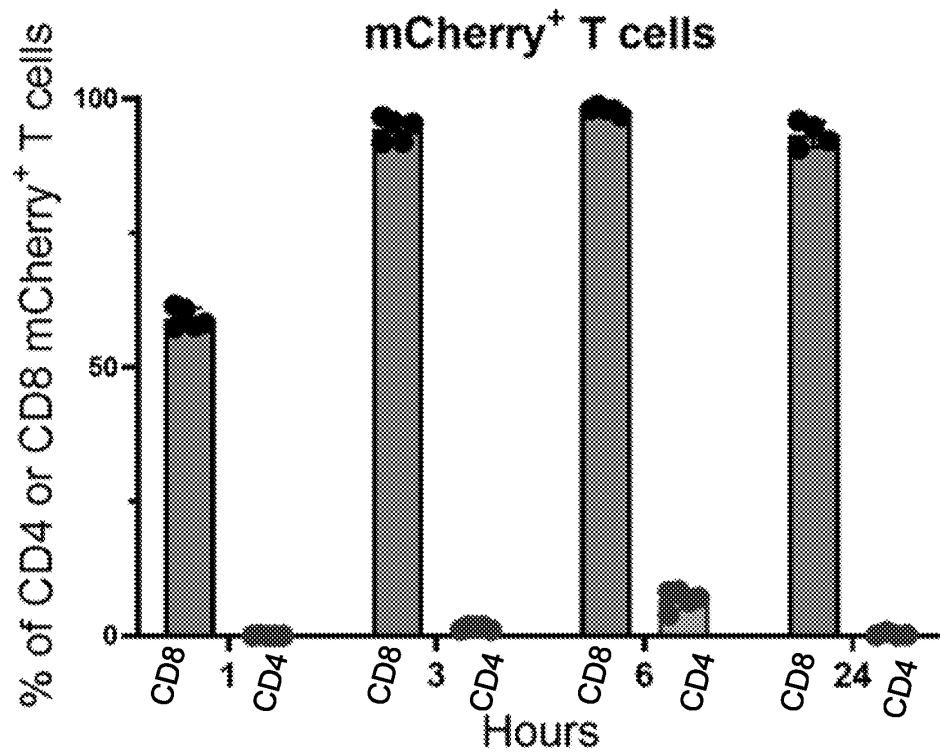


FIG. 23A

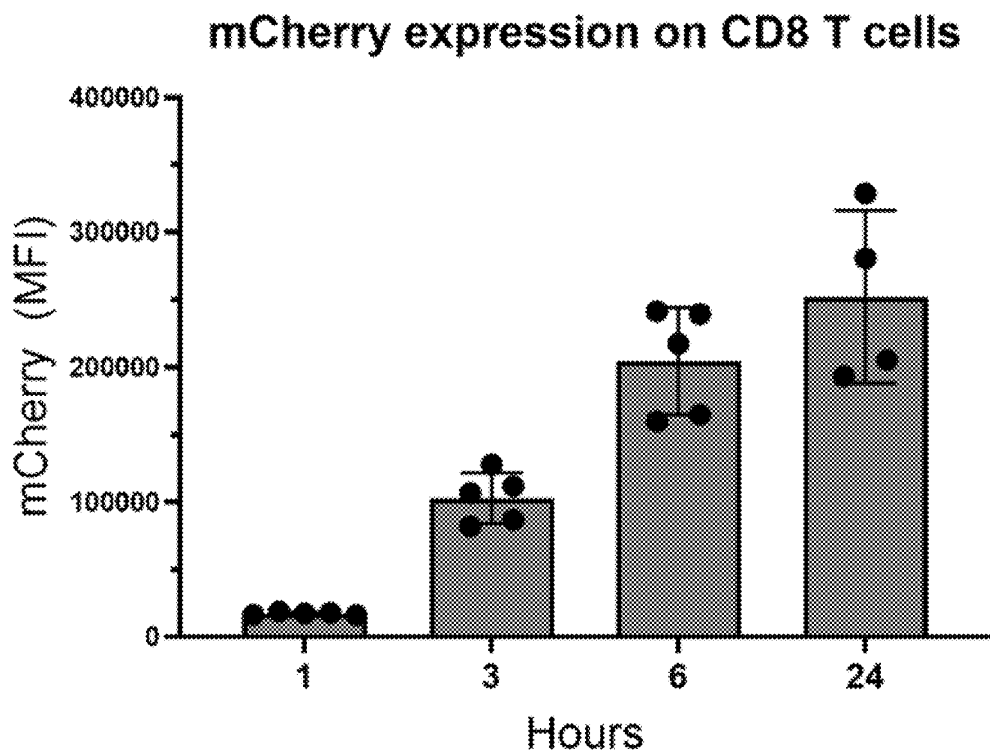


FIG. 23B

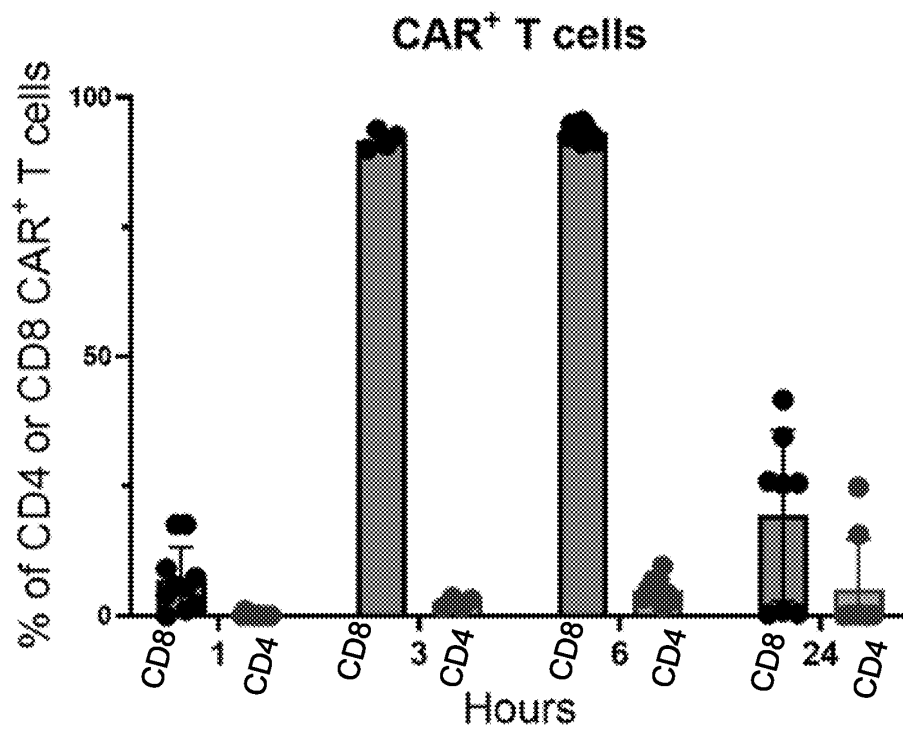


FIG. 23C

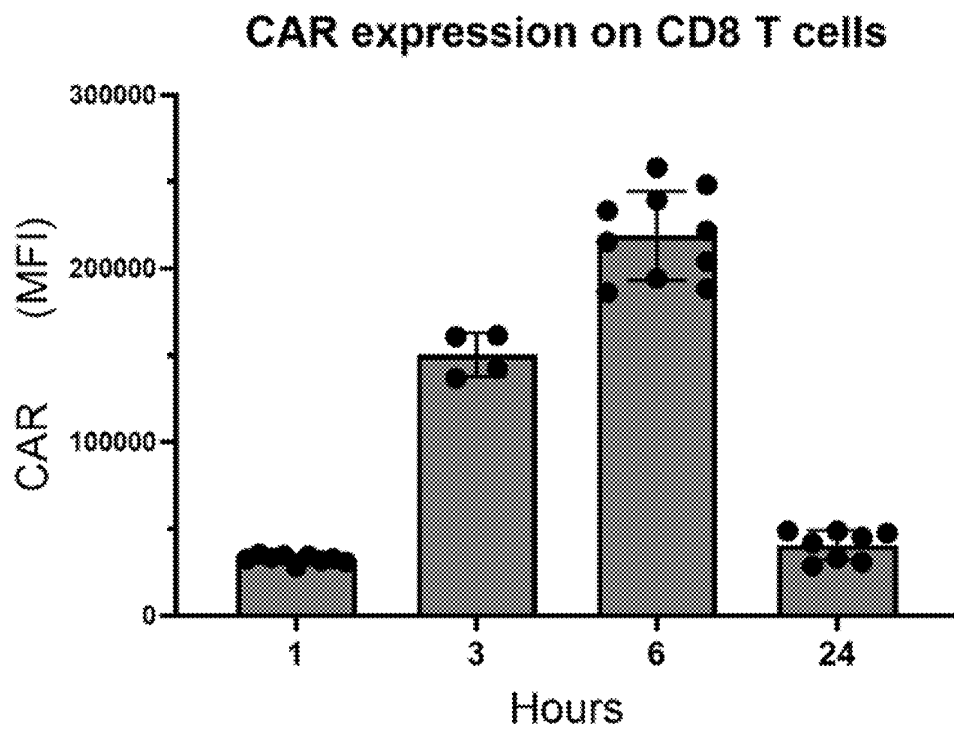
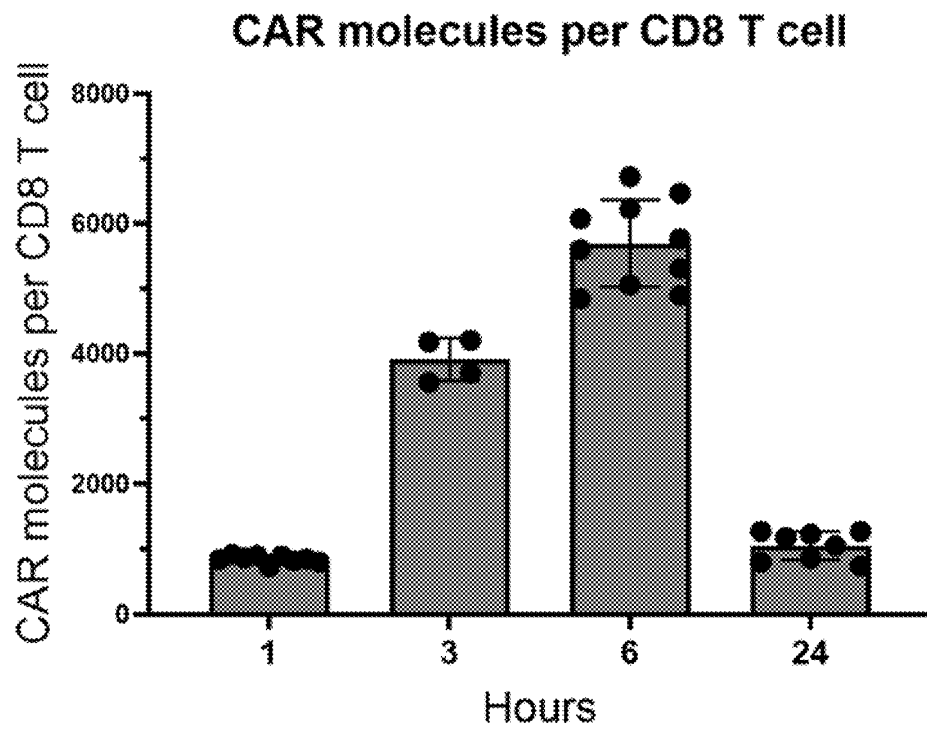
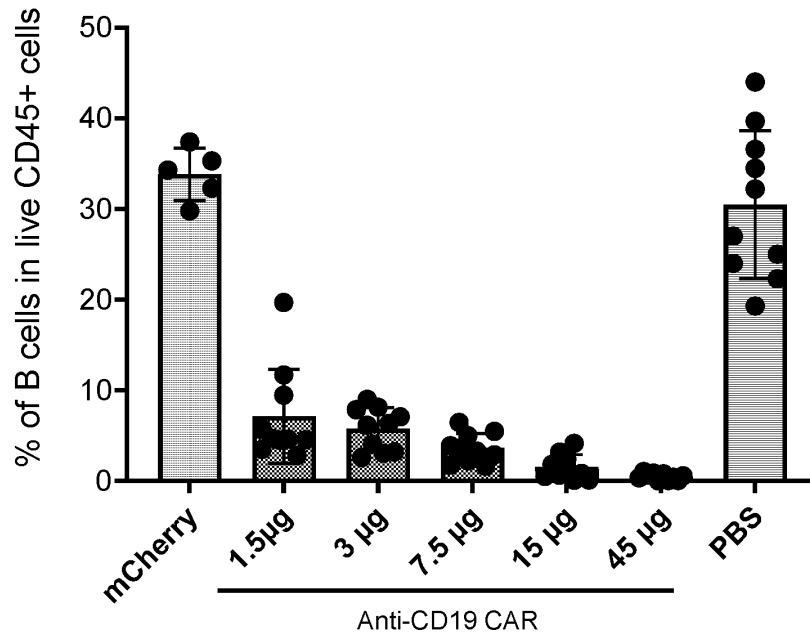
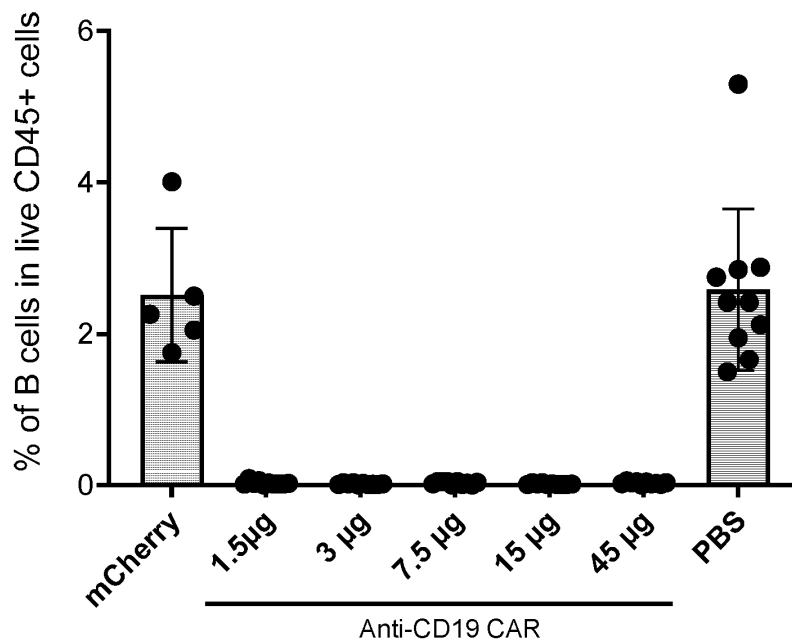
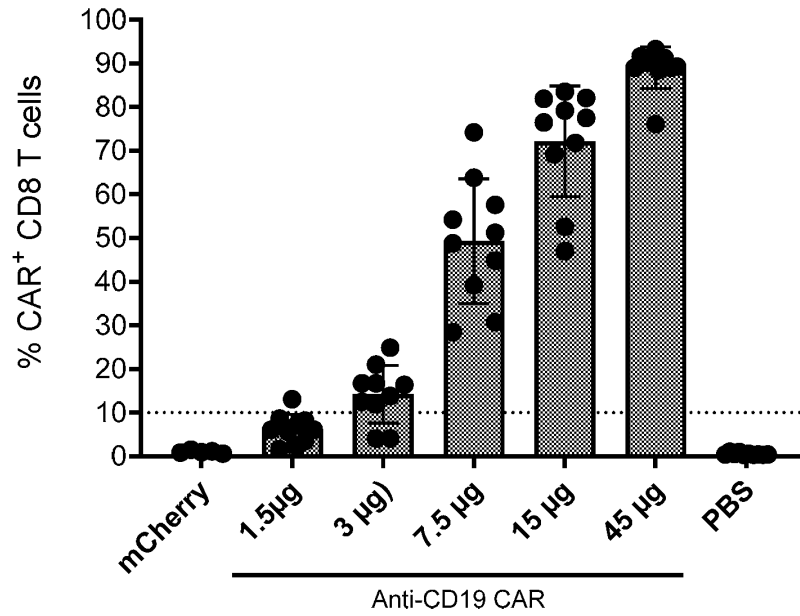


FIG. 23D

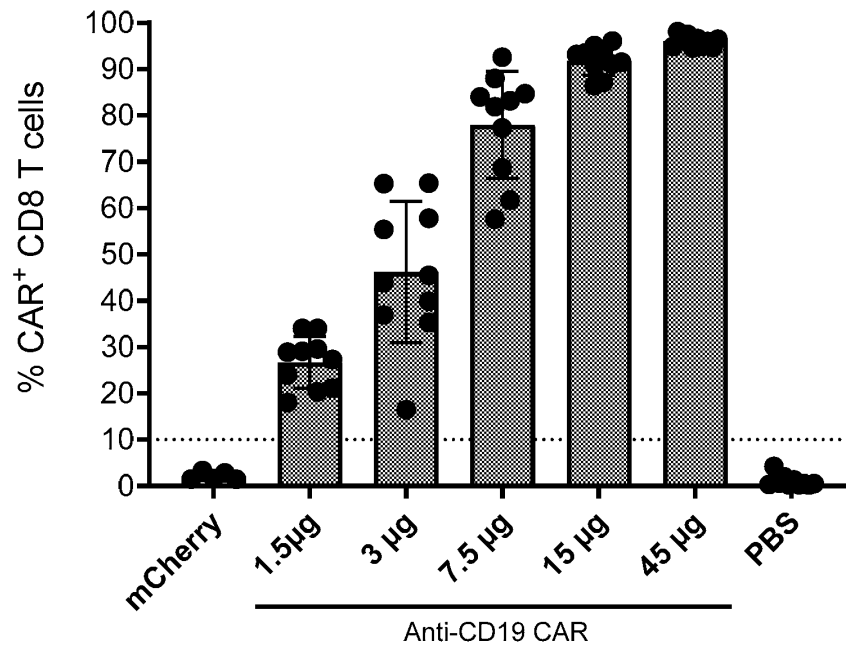
**FIG. 23E**

**B cell frequencies (Spleen)****B cell frequencies (Blood)****FIG. 24A**

### CAR expression in spleen



### CAR expression in blood

**FIG. 24B**

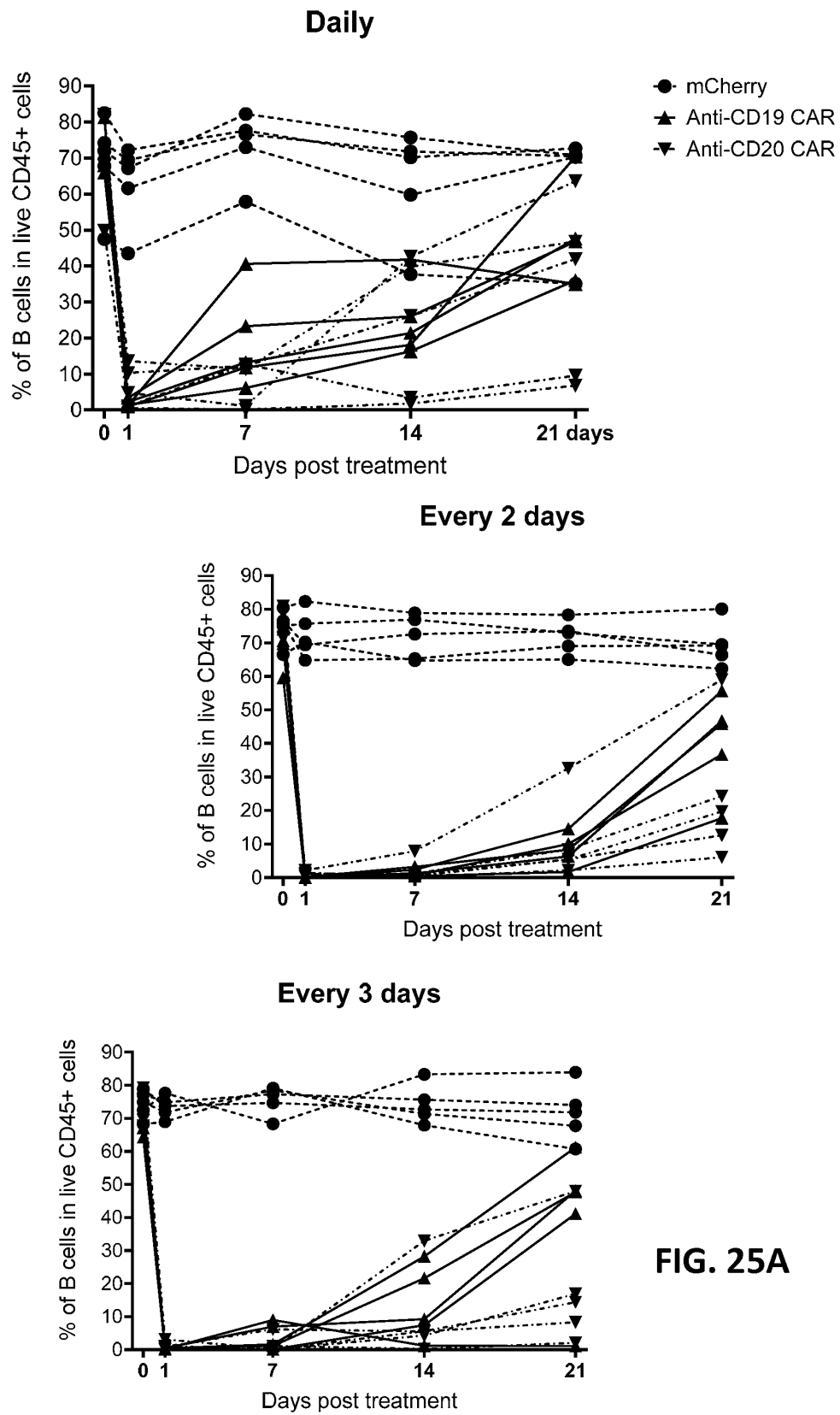


FIG. 25A

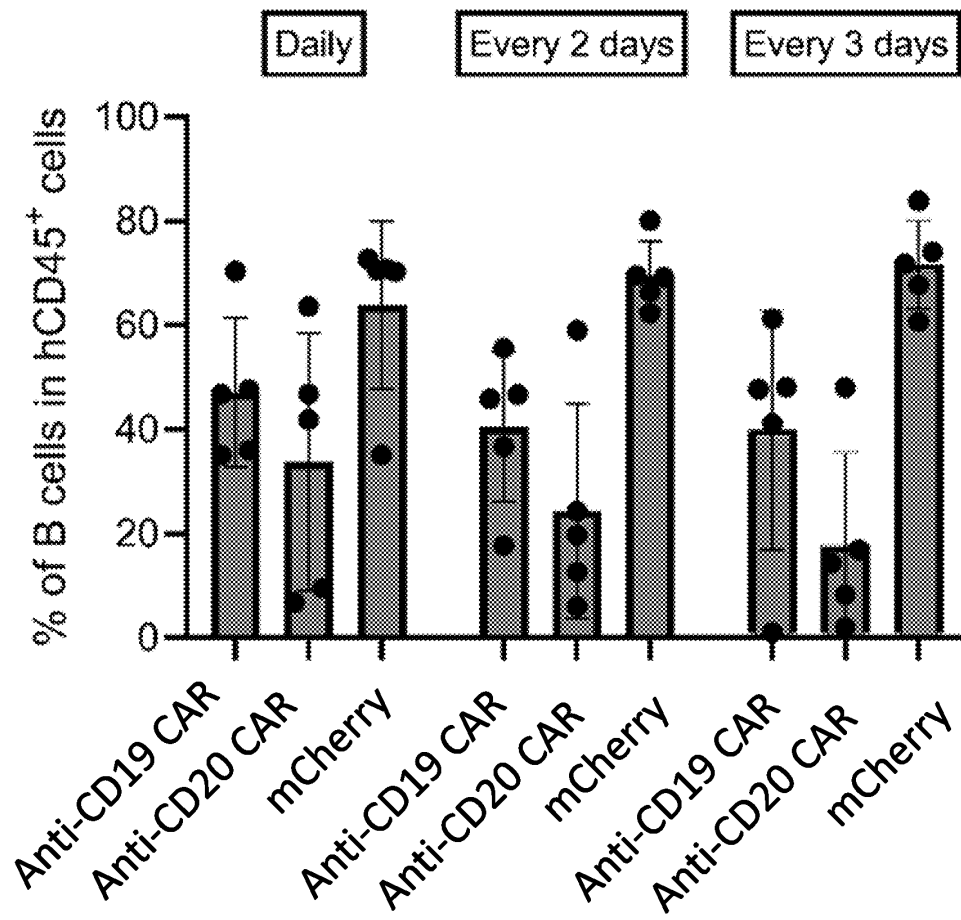


FIG. 25B

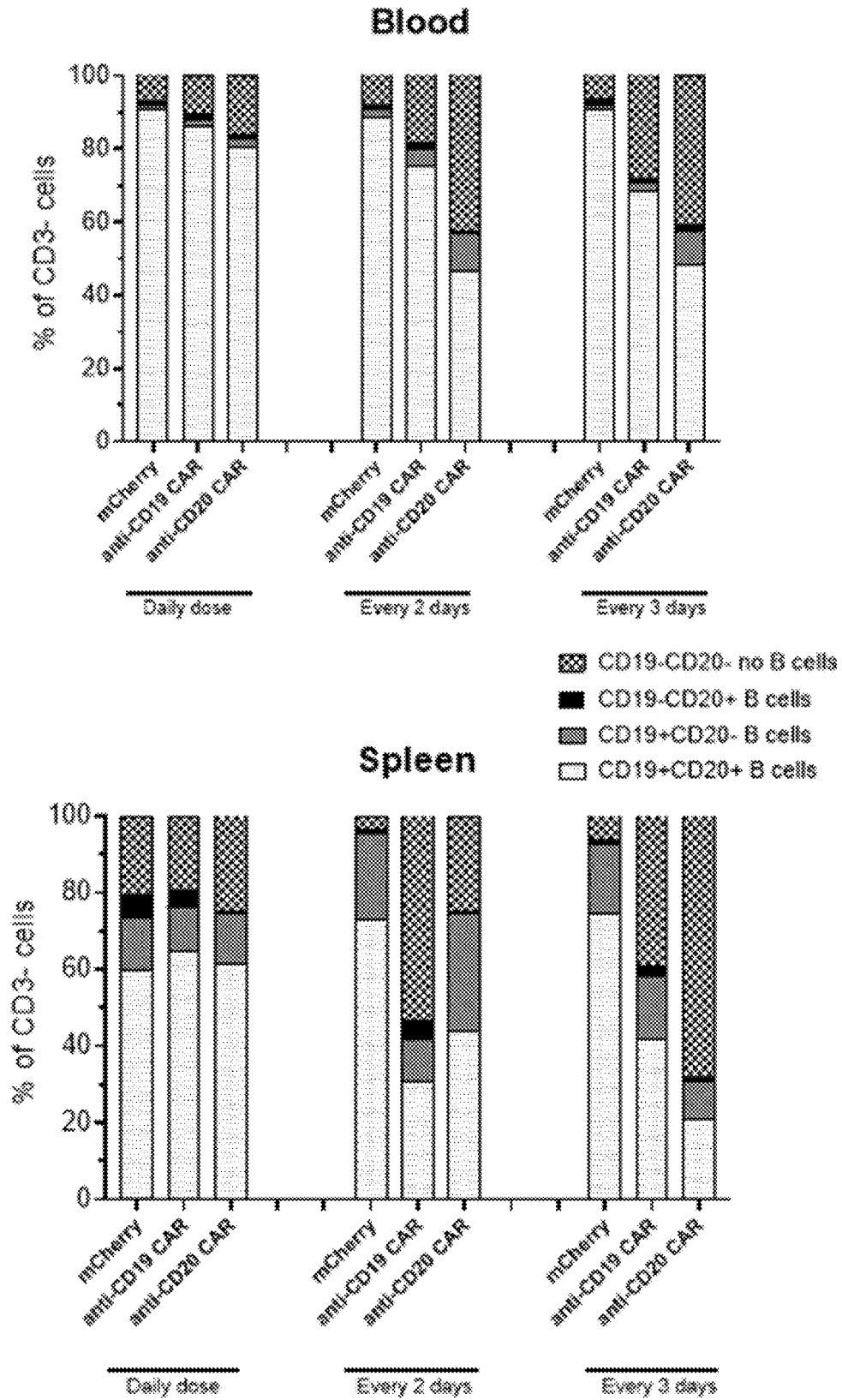
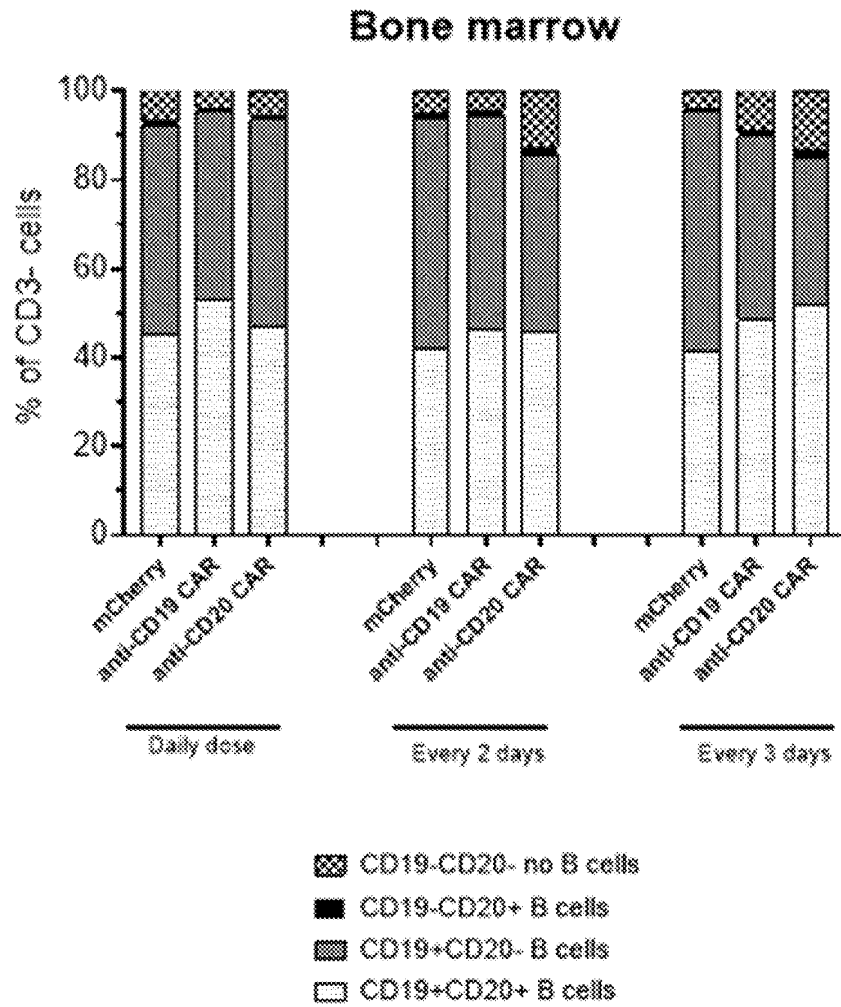
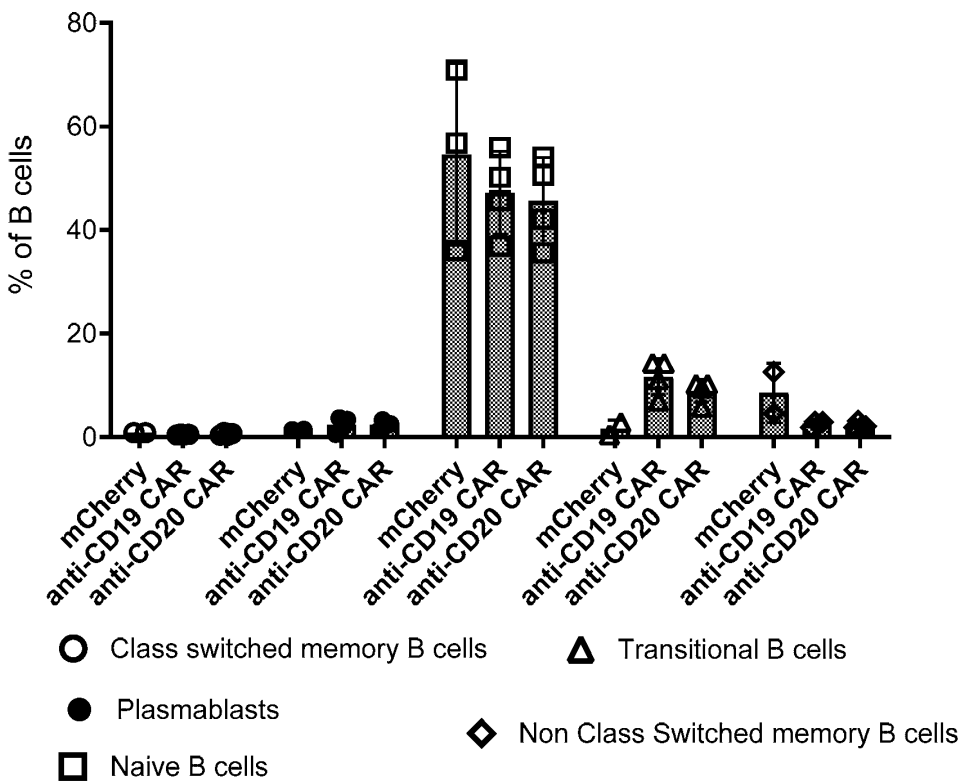


FIG. 26

**FIG. 26 (CONT'D)**

Spleen - Daily



Spleen - Every 2 days

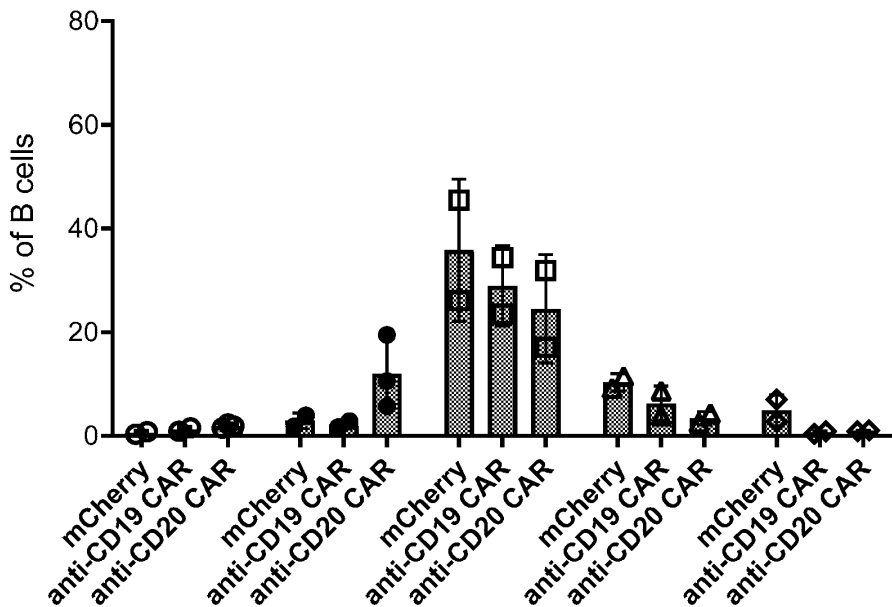


FIG. 27A

## Spleen - Every 3 days

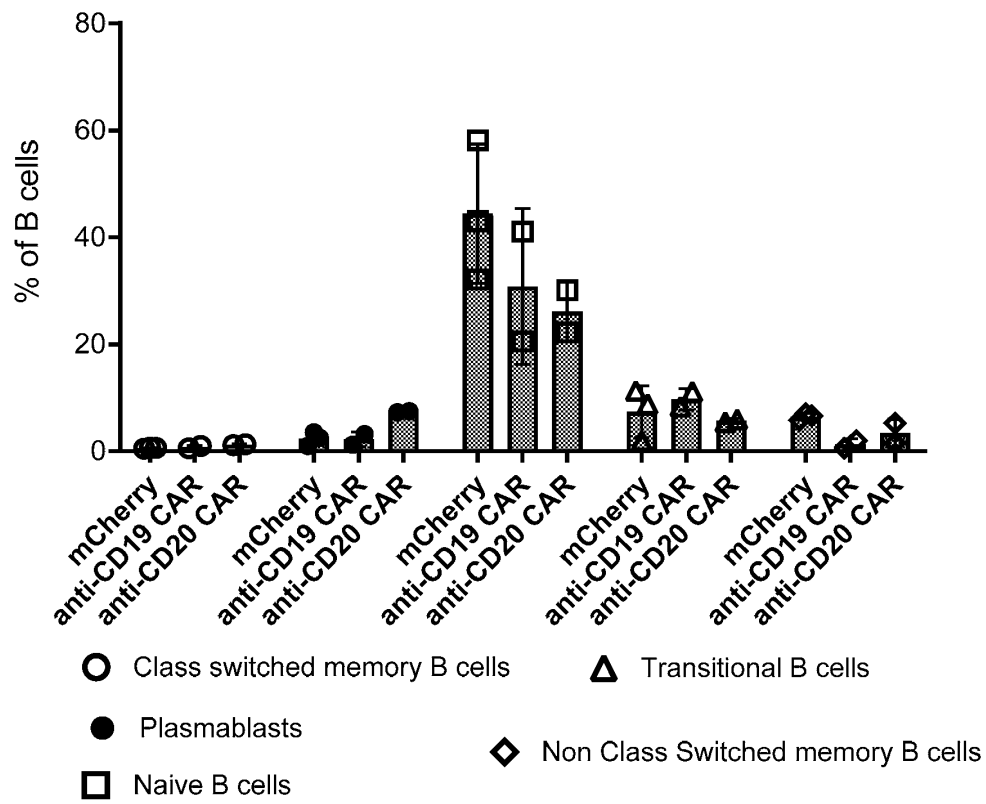
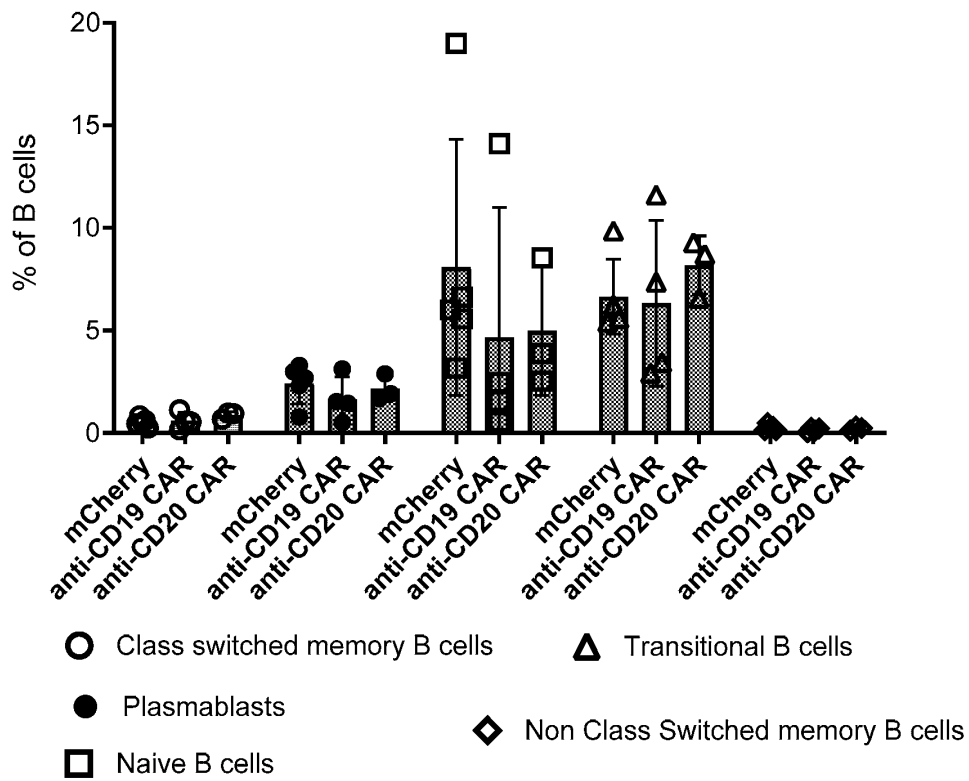
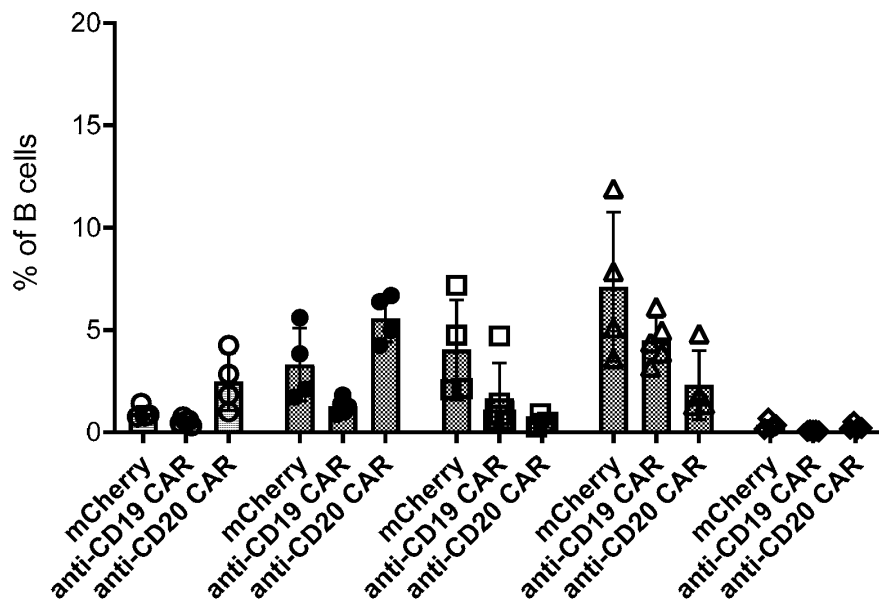


FIG. 27A (CONT'D)

**Bone marrow - Daily****Bone marrow - Every 2 days****FIG. 27B**

Bone marrow - Every 3 days

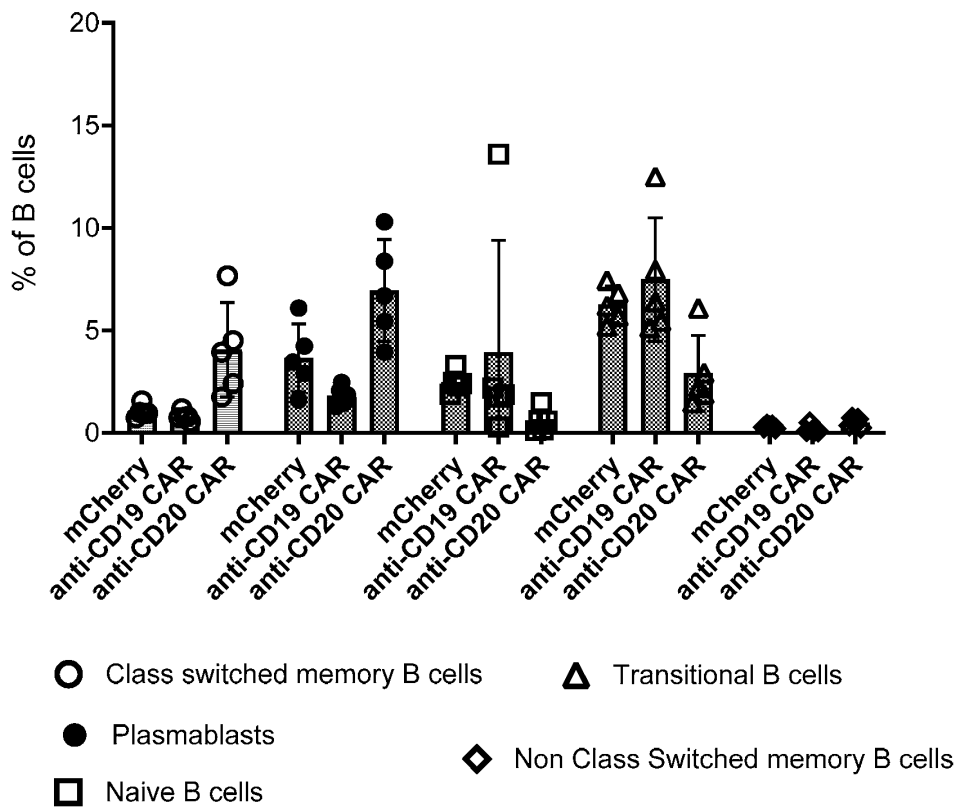


FIG. 27B (CONT'D)

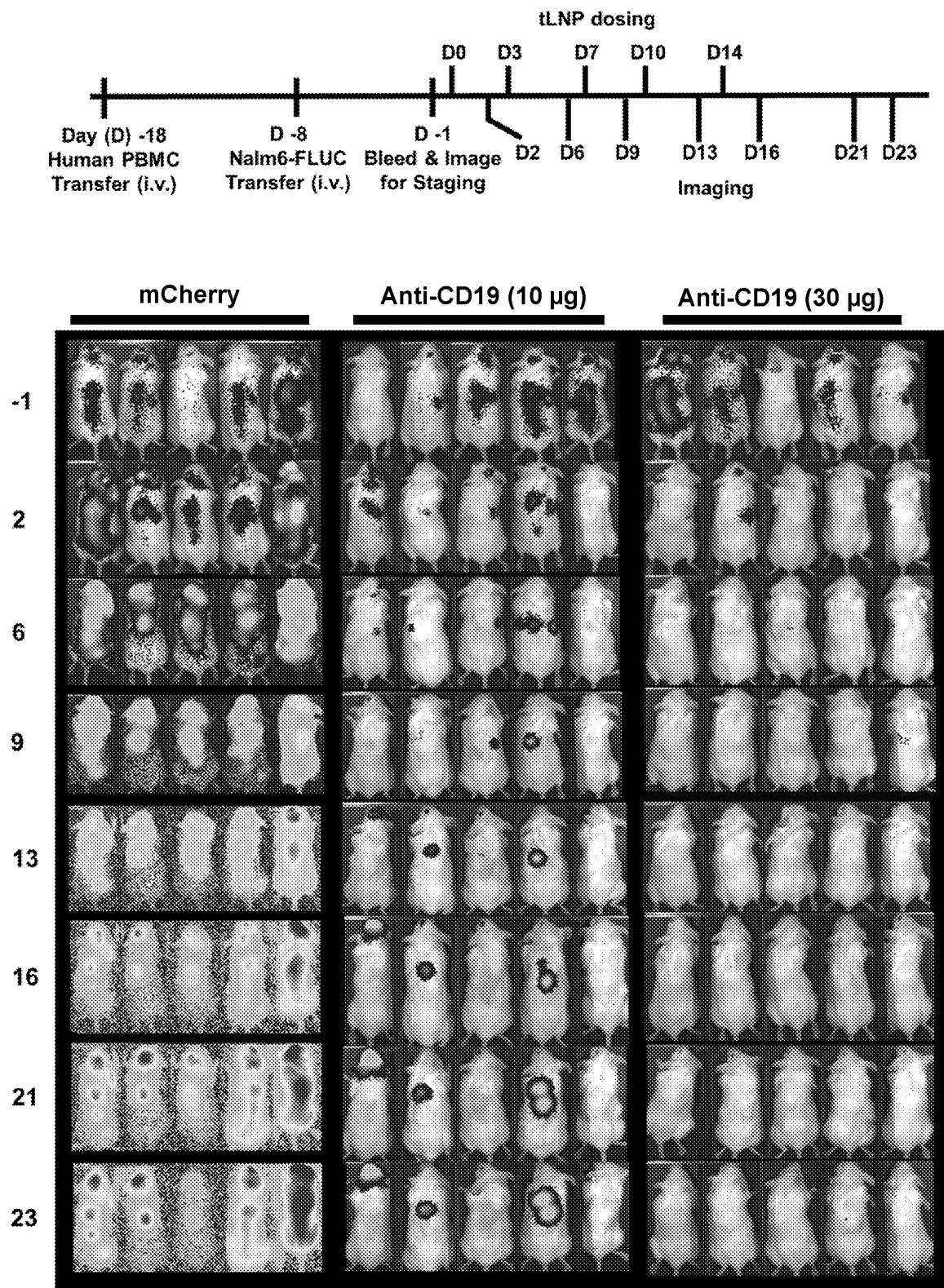


FIG. 28