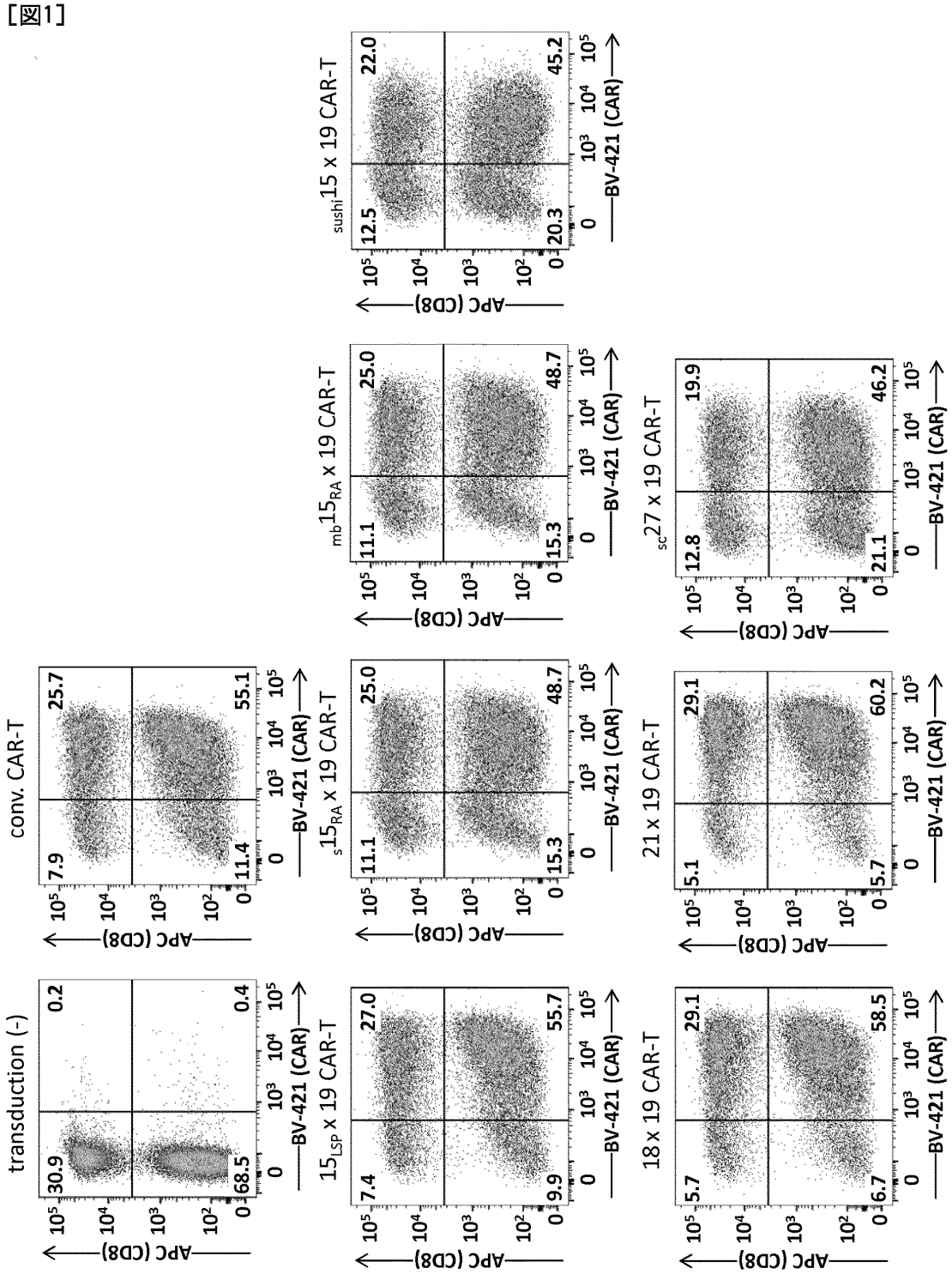
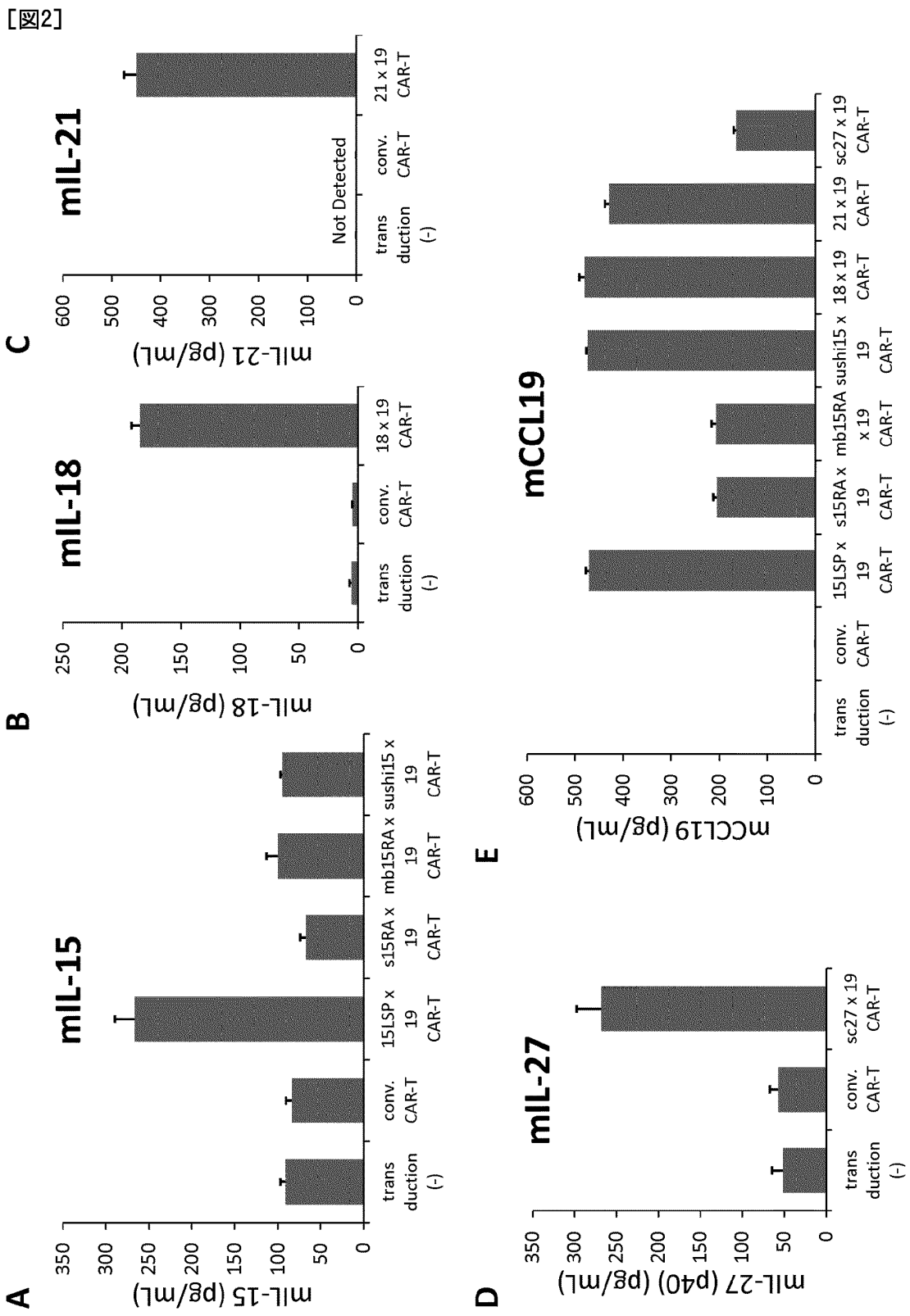
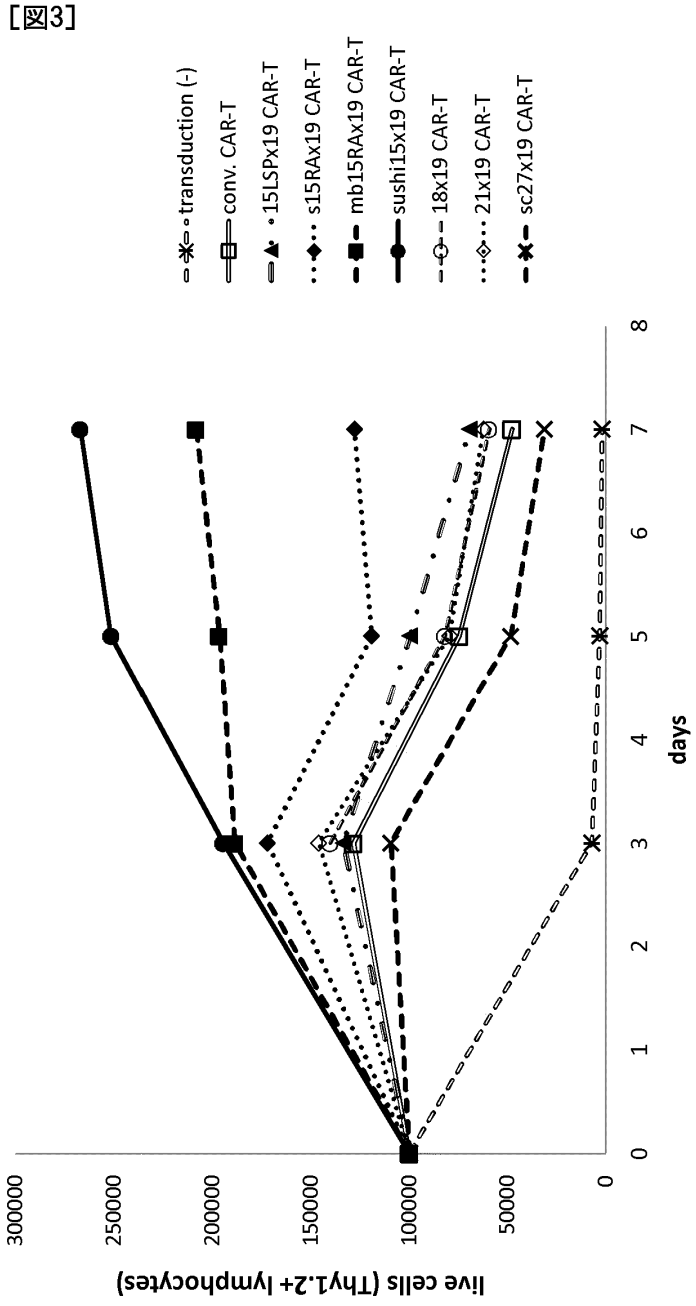
[図1]


transduction (-)
conv. CAR-T
$15_{LSP}$ x 19 CAR-T
$_{s}15_{RA}$ x 19 CAR-T
$_{mb}15_{RA}$ x 19 CAR-T
$_{sushi}$15 x 19 CAR-T
18 x 19 CAR-T
21 x 19 CAR-T
$_{sc}$27 x 19 CAR-T
APC (CD8)
BV-421 (CAR)
30.9
0.2
68.5
0.4
7.9
25.7
11.4
55.1
7.4
27.0
9.9
55.7
11.1
25.0
15.3
48.7
12.5
22.0
20.3
45.2
5.7
29.1
6.7
58.5
5.1
29.1
5.7
60.2
12.8
19.9
21.1
46.2

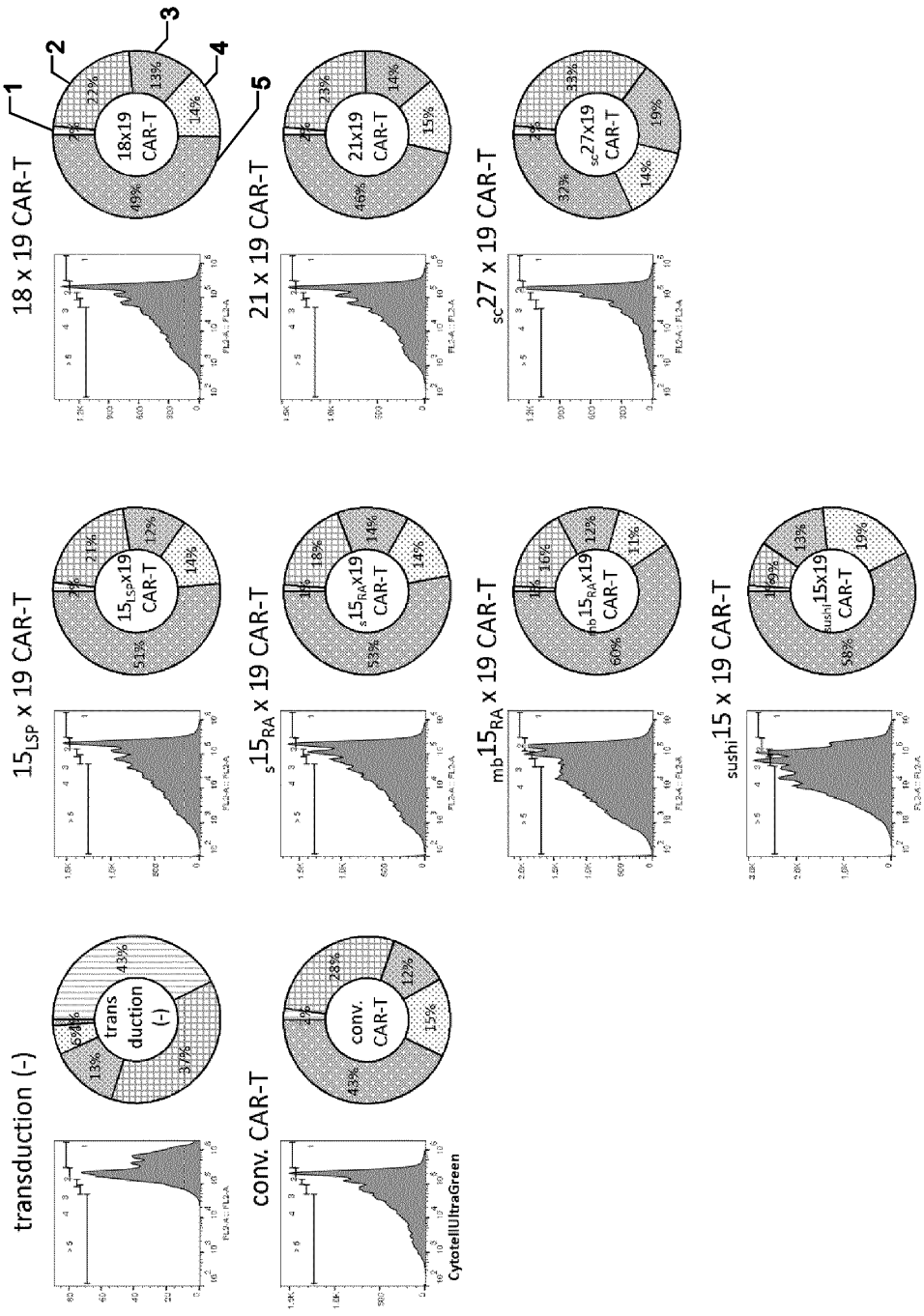
[図2]


A
mIL-15
mIL-15 (pg/mL)
0
50
100
150
200
250
300
350
trans duction (-)
conv. CAR-T
15LSP x 19 CAR-T
s15RA x 19 CAR-T
mb15RA x 19 CAR-T
sushi15 x 19 CAR-T
B
mIL-18
mIL-18 (pg/mL)
0
50
100
150
200
250
trans duction (-)
conv. CAR-T
18 x 19 CAR-T
C
mIL-21
mIL-21 (pg/mL)
0
100
200
300
400
500
600
Not Detected
trans duction (-)
conv. CAR-T
21 x 19 CAR-T
D
mIL-27
mIL-27 (p40) (pg/mL)
0
50
100
150
200
250
300
350
trans duction (-)
conv. CAR-T
sc27 x 19 CAR-T
E
mCCL19
mCCL19 (pg/mL)
0
100
200
300
400
500
600
trans duction (-)
conv. CAR-T
15LSP x 19 CAR-T
s15RA x 19 CAR-T
mb15RA x 19 CAR-T
sushi15 x 19 CAR-T
18 x 19 CAR-T
21 x 19 CAR-T
sc27 x 19 CAR-T

[図3]

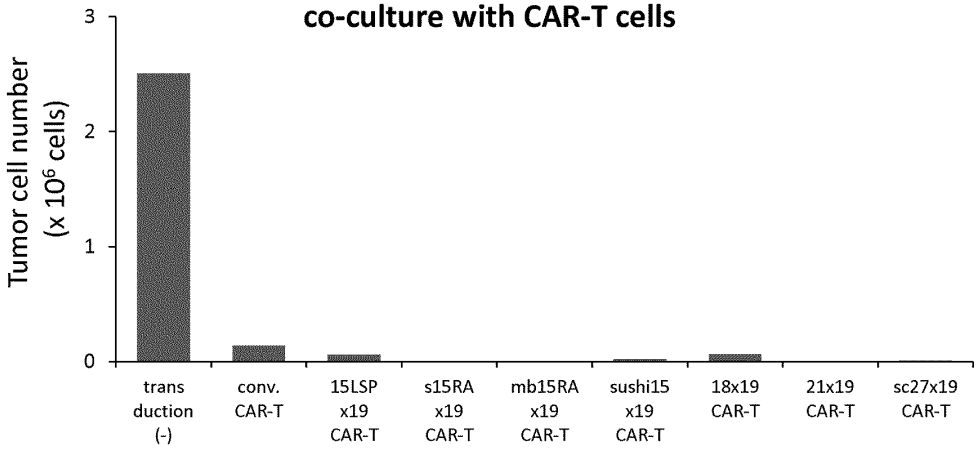

transduction (-)
conv. CAR-T
15LSPx19 CAR-T
s15RAx19 CAR-T
mb15RAx19 CAR-T
sushi15x19 CAR-T
18x19 CAR-T
21x19 CAR-T
sc27x19 CAR-T
live cells (Thy1.2+ lymphocytes)
300000
250000
200000
150000
100000
50000
0
0
1
2
3
4
5
6
7
8
days

[図4]

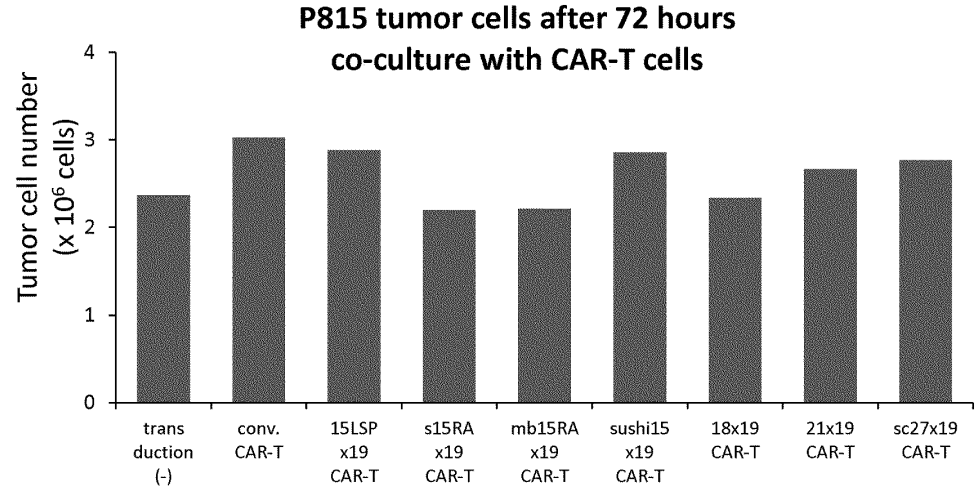

18 x 19 CAR-T
21 x 19 CAR-T
sc27 x 19 CAR-T
15LSP x 19 CAR-T
s15RA x 19 CAR-T
mb15RA x 19 CAR-T
sushi15 x 19 CAR-T
transduction (-)
conv. CAR-T
18x19 CAR-T
21x19 CAR-T
sc27x19 CAR-T
15LSPx19 CAR-T
sc15RAx19 CAR-T
mb15RAx19 CAR-T
sushi15x19 CAR-T
trans duction (-)
conv. CAR-T
1
2
3
4
5
CytotellUltraGreen

[図5]

A

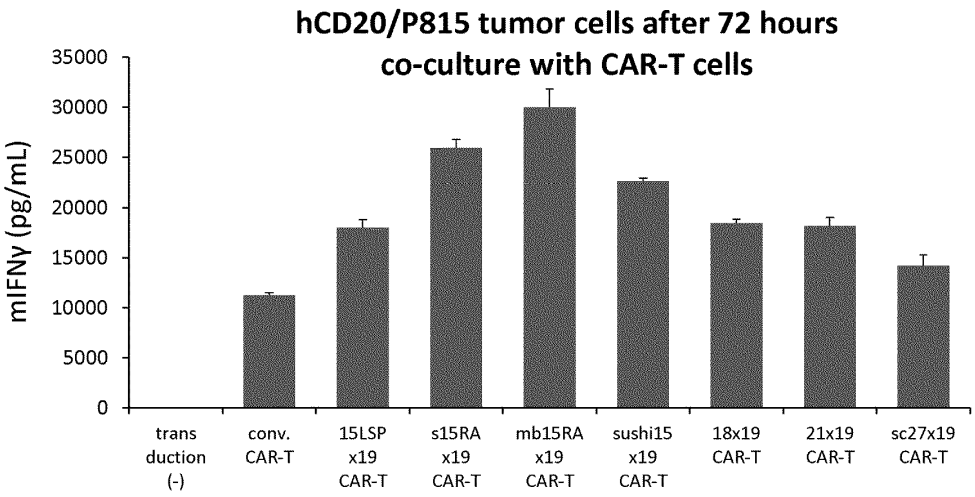

hCD20/P815 tumor cells after 72 hours
co-culture with CAR-T cells
Tumor cell number
(x 10^6 cells)
3
2
1
0
transduction (-)
conv. CAR-T
15LSP x19 CAR-T
s15RA x19 CAR-T
mb15RA x19 CAR-T
sushi15 x19 CAR-T
18x19 CAR-T
21x19 CAR-T
sc27x19 CAR-T

B

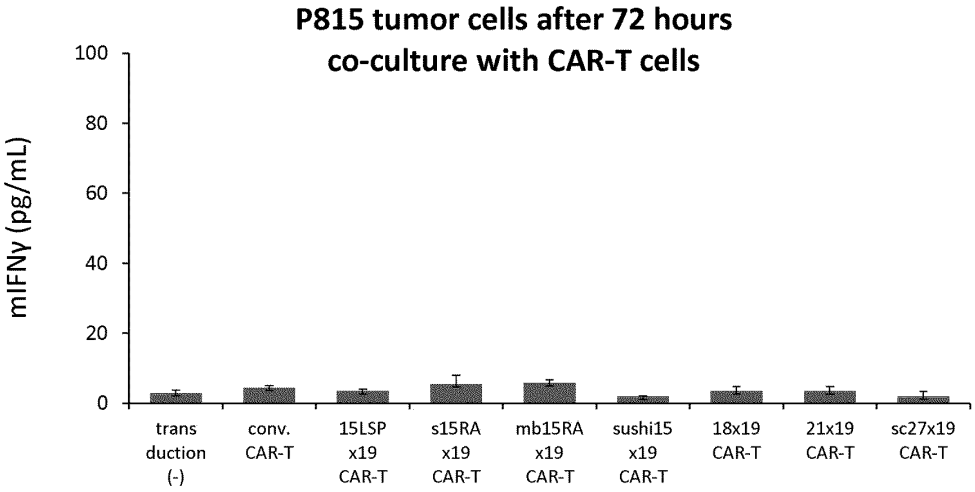

P815 tumor cells after 72 hours
co-culture with CAR-T cells
Tumor cell number
(x 10^6 cells)
4
3
2
1
0
transduction (-)
conv. CAR-T
15LSP x19 CAR-T
s15RA x19 CAR-T
mb15RA x19 CAR-T
sushi15 x19 CAR-T
18x19 CAR-T
21x19 CAR-T
sc27x19 CAR-T

[図6]

A


hCD20/P815 tumor cells after 72 hours
co-culture with CAR-T cells
mIFNγ (pg/mL)
35000
30000
25000
20000
15000
10000
5000
0
transduction (-)
conv. CAR-T
15LSP x19 CAR-T
s15RA x19 CAR-T
mb15RA x19 CAR-T
sushi15 x19 CAR-T
18x19 CAR-T
21x19 CAR-T
sc27x19 CAR-T

B


P815 tumor cells after 72 hours
co-culture with CAR-T cells
mIFNγ (pg/mL)
100
80
60
40
20
0
transduction (-)
conv. CAR-T
15LSP x19 CAR-T
s15RA x19 CAR-T
mb15RA x19 CAR-T
sushi15 x19 CAR-T
18x19 CAR-T
21x19 CAR-T
sc27x19 CAR-T

[図7]

配列番号１：抗ヒトCD20 CAR DNA断片（DNA断片 #1）

anti-human CD20 scFv

CCATGGACTGGACCTGGCGGATCCTGTTCCTGGTGGCTGCTGCTACAGGCGCCCACAGCC

Nco I

AGATCGTGCTGTCTCAGTCTCCCGCCATCCTGTCTGCTAGCCCTGGCGAGAAAGTGACCA

TGACCTGCAGAGCCAGCAGCAGCGTGTCCTACATCCACTGGTTCCAGCAGAAGCCCGGCA

GCAGCCCCAAGCCTTGGATCTACGCCACAAGCAACCTGGCCTCTGGCGTGCCAGTGCGGT

TTAGCGGCTCTGGCTCTGGCACCAGCTACAGCCTGACCATCAGCAGAGTGGAAGCCGAGG

ACGCCGCCACCTACTACTGTCAGCAGTGGACCAGCAACCCCCCCACATTCGGCGGAGGCA

CCAAGCTGGAAATCAAGGGCGGAGGCGGATCTGGCGGCGGAGGATCTGGGGGACGCGGCT

CTCAGGTGCAGCTGCAGCAGCCTGGCGCTGAGCTCGTGAAACCTGGCGCCTCCGTGAAGA

TGAGCTGCAAGGCCAGCGGCTACACCTTCACAAGCTACAACATGCACTGGGTCAAGCAGA

CCCCTGGCAGAGGCCTGGAATGGATCGGCGCTATCTACCCCGGCAACGGCGACACCTCCT

ACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGCCGACAAGAGCAGCAGCACAGCCT

ACATGCAGCTGTCCTCCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGATCTA

CCTACTACGGCGGCGACTGGTACTTCAACGTGTGGGGCGCTGGCACCACCGTGACCGTGT

3rd TM domain (CD8)

CTGCTGCGGCCGCAGTCGTGCCAGTCCTTCAGAAAGTGAACTCTACTACTACCAAGCCAG

TGCTGCGAACTCCCTCACCTGTGCACCCTACCGGGACATCTCAGCCCCAGAGACCAGAAG

ATTGTCGGCCCCGTGGCTCAGTGAAGGGGACCGGATTGGACTTCGCCTGTGATATTTACA

TCTGGGCACCCTTGGCCGGAATCTGCGTGGCCCCTCTGCTGTCCTTGATCATCACTCTCA

CP domain (CD28_4-1BB_CD3z)

TCTGCTACCACAGGAGCCGAAATAGTAGAAGGAACAGACTCCTTCAAAGTGACTACATGA

ACATGACTCCCCGGAGGCCTGGGCTCACTCGAAAGCCTTACCAGCCCTACGCCCCTGCCA

GAGACTTTGCAGCGTACCGCCCCAAATGGATCAGGAAAAAATTCCCCCACATATTCAAGC

AACCATTTAAGAAGACCACTGGAGCAGCTCAAGAGGAAGATGCTTGTAGCTGCCGATGTC

CACAGGAAGAAGAAGGAGGAGGAGGAGGCTATGAGCTGAGAGCAAAATTCAGCAGGAGTG

CAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCTCAATCTAGGGC

Sbf I

GAAGAGAGGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCA

AACAGCAGAGGAGGAGGAACCCCCAGGAAGGCGTATACAATGCACTGCAGAAAGACAAGA

TGGCAGAAGCCTACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGAGGCAAGGGGCACG

ATGGCCTTTACCAGGGTCTCAGCACTGCCACCAAGGACACCTATGATGCCCTGCATATGC

AGACCCTGGCCCCTCGC

[図8]

配列番号３：終止コドンおよび制限酵素サイトをコードするMCS DNA断片（DNA断片 #2）

TGAAAGCTTAAACCAGTTAACTGGAAAACGCGTAAAGTCGAC

Stop codon

Sal I

[図9]

配列番号４:$IL15_{LSP}$_F2A_CCL19 DNA断片（DNA断片 #3）

F2A

GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC

Mouse IL-15 long signal peptide

GTGGAGAGCAACCCTGGCCCTATGAAGATCCTGAAGCCCTACATGAGGAACACCAGCATC

Mouse IL-15

TCCTGTTACCTCTGTTTCCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTG

TTCATCCTGGGCTGCGTGTCCGTGGGCCTGCCTAAGACCGAGGCCAACTGGATCGACGTG

AGATACGACCTGGAGAAGATCGAGAGCCTGATCCAGAGCATCCACATCGACACCACACTG

TACACCGACAGCGACTTCCACCCCAGCTGCAAGGTGACCGCCATGAACTGCTTTCTGCTG

GAGCTGCAGGTGATCCTGCACGAGTACAGCAACATGACCCTGAACGAGACCGTCAGAAAC

GTGCTGTACCTGGCCAACTCCACCCTGAGCAGCAACAAGAACGTGGCCGAGAGCGGCTGC

AAGGAGTGCGAGGAGCTCGAGGAGAAAACCTTCACCGAGTTCCTGCAGAGCTTCATCAGG

F2A

ATCGTCCAGATGTTCATCAACACCAGCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGAC

Mouse CCL19

CTGCTGAAGCTGGCCGGAGACGTGGAGAGCAACCCTGGCCCATGCATGGCCCCCAGGGTG

ACCCCTCTGCTGGCCTTCAGCCTGCTGGTGCTGTGGACCTTCCCTGCCCCTACCCTGGGA

GGAGCCAATGACGCCGAGGACTGCTGCCTGAGCGTGACCCAGAGACCCATCCCCGGCAAC

ATCGTGAAGGCCTTCAGGTACCTGCTGAACGAGGACGGCTGCAGAGTGCCTGCCGTGGTG

TTCACCACCCTGAGGGGCTACCAGCTGTGTGCTCCCCCTGACCAGCCTTGGGTGGACAGG

ATCATCAGGAGGCTGAAGAAGAGCAGCGCCAAGAACAAGGGCAACAGCACCAGGAGGAGC

CCCGTGAGCTGAAAGCTTAAACCAGTTAACTGGAAAACGCGTAAAGTCGAC

Stop codon

Sal I

[図10]

配列番号６：$_{s}$IL15$_{RA}$_F2A_CCL19 DNA断片（DNA断片 #4）

F2A
GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC
Mouse IL-2 signal peptide
GTGGAGAGCAACCCTGGCCCTATGTATTCCATGCAGCTGGCCAGCTGTGTGACCCTGACA
Mouse IL-15
CTGGTGCTGCTGGTGAACAGCGGCATCCACGTGTTCATTCTGGGATGCGTCTCCGTGGGC
CTCCCCAAGACCGAGGCTAACTGGATCGACGTCAGGTACGACCTGGAGAAGATCGAAAGC
CTGATCCAGTCCATCCACATCGATACAACCCTGTACACAGATTCCGACTTCCACCCCTCC
TGTAAGGTCACCGCCATGAACTGCTTCCTCCTGGAACTCCAGGTCATCCTGCACGAGTAC
TCCAACATGACCCTGAACGAAACCGTGAGGAATGTGCTGTACCTCGCCAACTCCACACTG
AGCTCCAATAAGAACGTGGCTGAGAGCGGCTGCAAGGAGTGTGAGGAGCTGGAAGAGAAG
ACCTTCACCGAGTTCCTGCAGAGCTTCATCAGGATCGTGCAGATGTTCATCAACACAAGC
26aa linker
TCCGGCGGAGGATCCGGAGGAGGAGGAAGCGGAGGCGGCGGCTCCGGAGGCGGAGGAAGC
Mouse IL-15RA Extracellular domain
GGAGGAGGAAGCCTGCAAGGAACCACCTGTCCCCCTCCCGTGTCCATCGAGCACGCCGAC
ATCAGGGTGAAGAACTACTCCGTCAACAGCAGGGAAAGGTACGTGTGCAACTCCGGCTTT
AAGAGGAAGGCCGGCACCAGCACCCTCATCGAGTGCGTGATCAACAAGAACACAAACGTG
GCCCACTGGACCACCCCCAGCCTGAAGTGTATTAGAGACCCCAGCCTGGCTCATTACAGC
CCCGTGCCCACAGTGGTGACACCCAAGGTGACAAGCCAGCCTGAGAGCCCTAGCCCTAGC
GCCAAAGAGCCCGAGGCCTTTTCCCCCAAGTCCGACACCGCCATGACCACCGAGACAGCC
ATCATGCCTGGCAGCAGACTGACCCCTAGCCAGACCACATCCGCTGGCACCACAGGCACC
GGCTCCCACAAGAGCTCCAGAGCCCCTAGCCTGGCTGCCACAATGACACTGGAACCTACC
F2A
GCCTCCACCAGCCTCAGAATCACAGAGATCTCCCCCCACAGCAGCAAGATGACCAAGGGC
AGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGACGTGGAGAGC
Mouse CCL19
AACCCTGGCCCATGCATGGCCCCCAGGGTGACCCCTCTGCTGGCCTTCAGCCTGCTGGTG
CTGTGGACCTTCCCTGCCCCTACCCTGGGAGGAGCCAATGACGCCGAGGACTGCTGCCTG
AGCGTGACCCAGAGACCCATCCCCGGCAACATCGTGAAGGCCTTCAGGTACCTGCTGAAC
GAGGACGGCTGCAGAGTGCCTGCCGTGGTGTTCACCACCCTGAGGGGCTACCAGCTGTGT
GCTCCCCCTGACCAGCCTTGGGTGGACAGGATCATCAGGAGGCTGAAGAAGAGCAGCGCC
AAGAACAAGGGCAACAGCACCAGGAGGAGCCCCGTGAGCTGAAAGCTTAAACCAGTTAAC
Stop codon
TGGAAAACGCGTAAAGTCGAC
Sal I

[図11]

配列番号８：$_{mb}IL15_{RA}$_F2A_CCL19 DNA断片（DNA断片 #5）

F2A
GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC
Mouse IL-2 signal peptide
GTGGAGAGCAACCCTGGCCCTATGTATTCCATGCAGCTGGCCAGCTGTGTGACCCTGACA
Mouse IL-15
CTGGTGCTGCTGGTGAACAGCGGCATCCACGTGTTCATTCTGGGATGCGTCTCCGTGGGC
CTCCCCAAGACCGAGGCTAACTGGATCGACGTCAGGTACGACCTGGAGAAGATCGAAAGC
CTGATCCAGTCCATCCACATCGATACAACCCTGTACACAGATTCCGACTTCCACCCCTCC
TGTAAGGTCACCGCCATGAACTGCTTCCTCCTGGAACTCCAGGTCATCCTGCACGAGTAC
TCCAACATGACCCTGAACGAAACCGTGAGGAATGTGCTGTACCTCGCCAACTCCACACTG
AGCTCCAATAAGAACGTGGCTGAGAGCGGCTGCAAGGAGTGTGAGGAGCTGGAAGAGAAG
ACCTTCACCGAGTTCCTGCAGAGCTTCATCAGGATCGTGCAGATGTTCATCAACACAAGC
26aa linker
TCCGGCGGAGGATCCGGAGGAGGAGGAAGCGGAGGCGGCGGCTCCGGAGGCGGAGGAAGC
Mouse IL-15RA
GGAGGAGGAAGCCTGCAAGGAACCACCTGTCCCCCTCCCGTGTCCATCGAGCACGCCGAC
ATCAGGGTGAAGAACTACTCCGTCAACAGCAGGGAAAGGTACGTGTGCAACTCCGGCTTT
AAGAGGAAGGCCGGCACCAGCACCCTCATCGAGTGCGTGATCAACAAGAACACAAACGTG
GCCCACTGGACCACCCCCAGCCTGAAGTGTATTAGAGACCCCAGCCTGGCTCATTACAGC
CCCGTGCCCACAGTGGTGACACCCAAGGTGACAAGCCAGCCTGAGAGCCCTAGCCCTAGC
GCCAAAGAGCCCGAGGCCTTTTCCCCCAAGTCCGACACCGCCATGACCACCGAGACAGCC
ATCATGCCTGGCAGCAGACTGACCCCTAGCCAGACCACATCCGCTGGCACCACAGGCACC
GGCTCCCACAAGAGCTCCAGAGCCCCTAGCCTGGCTGCCACAATGACACTGGAACCTACC
GCCTCCACCAGCCTCAGAATCACAGAGATCTCCCCCCACAGCAGCAAGATGACCAAGGTG
GCCATCTCCACCTCCGTGCTGCTGGTGGGAGCCGGCGTGGTCATGGCCTTTCTGGCCTGG
TACATCAAGTCCAGGCAGCCTAGCCAACCTTGCAGGGTGGAGGTGGAAACTATGGAAACC
F2A
GTGCCCATGACCGTGAGGGCCAGCTCCAAGGAGGATGAAGACACCGGCGCCGGCAGCGGC
GTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGACGTGGAGAGCAACCCT
Mouse CCL19
GGCCCATGCATGGCCCCCAGGGTGACCCCTCTGCTGGCCTTCAGCCTGCTGGTGCTGTGG
ACCTTCCCTGCCCCTACCCTGGGAGGAGCCAATGACGCCGAGGACTGCTGCCTGAGCGTG
ACCCAGAGACCCATCCCCGGCAACATCGTGAAGGCCTTCAGGTACCTGCTGAACGAGGAC
GGCTGCAGAGTGCCTGCCGTGGTGTTCACCACCCTGAGGGGCTACCAGCTGTGTGCTCCC
CCTGACCAGCCTTGGGTGGACAGGATCATCAGGAGGCTGAAGAAGAGCAGCGCCAAGAAC
AAGGGCAACAGCACCAGGAGGAGCCCCGTGAGCTGAAAGCTTAAACCAGTTAACTGGAAA
Stop codon
ACGCGTAAAGTCGAC
Sal I

[図12]

配列番号10：sushiIL15_F2A_CCL19 DNA断片（DNA断片 #6）

F2A

GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC

Mouse IL-15RAsushi

GTGGAGAGCAACCCTGGCCCTATGGCCTCCCCTCAGCTGAGAGGCTACGGCGTGCAGGCT

ATCCCCGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCCTCTGAGAGTGACCCCTGGC

ACAACCTGCCCTCCTCCCGTGAGCATCGAGCACGCCGACATCAGGGTGAAGAACTACTCC

GTGAACAGCAGGGAGAGGTACGTGTGCAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGC

ACCCTGATCGAGTGCGTGATCAACAAGAACACCAACGTGGCCCACTGGACCACCCCCAGC

26aa linker

CTGAAGTGCATCAGGAGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCAGCGGCGGC

Mouse IL-15

GGCGGCAGCCTGCAGAACTGGATCGACGTCAGGTACGACCTGGAGAAGATCGAAAGCCTG

ATCCAGTCCATCCACATCGATACAACCCTGTACACAGATTCCGACTTCCACCCCTCCTGT

AAGGTCACCGCCATGAACTGCTTCCTCCTGGAACTCCAGGTCATCCTGCACGAGTACTCC

AACATGACCCTGAACGAAACCGTGAGGAATGTGCTGTACCTCGCCAACTCCACACTGAGC

TCCAATAAGAACGTGGCTGAGAGCGGCTGCAAGGAGTGTGAGGAGCTGGAAGAGAAGACC

F2A

TTCACCGAGTTCCTGCAGAGCTTCATCAGGATCGTGCAGATGTTCATCAACACAAGCGGC

AGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGACGTGGAGAGC

Mouse CCL19

AACCCTGGCCCATGCATGGCCCCCAGGGTGACCCCTCTGCTGGCCTTCAGCCTGCTGGTG

CTGTGGACCTTCCCTGCCCCTACCCTGGGAGGAGCCAATGACGCCGAGGACTGCTGCCTG

AGCGTGACCCAGAGACCCATCCCCGGCAACATCGTGAAGGCCTTCAGGTACCTGCTGAAC

GAGGACGGCTGCAGAGTGCCTGCCGTGGTGTTCACCACCCTGAGGGGCTACCAGCTGTGT

GCTCCCCCTGACCAGCCTTGGGTGGACAGGATCATCAGGAGGCTGAAGAAGAGCAGCGCC

AAGAACAAGGGCAACAGCACCAGGAGGAGCCCCGTGAGCTGAAAGCTTAAACCAGTTAAC

Stop codon

TGGAAAACGCGTAAAGTCGAC

Sal I

[図13]

配列番号１２:IL-18_F2A_CCL19 DNA断片(DNA断片 #7)

F2A

GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC

Mouse IL-18

GTGGAGAGCAACCCTGGCCCTATGGCCGCCATGAGCGAGGACTCCTGCGTGAACTTTAAG

GAGATGATGTTCATCGACAACACCCTGTACTTCATCCCCGAGGAAAACGGCGACCTGGAG

AGCGACAACTTCGGCAGGCTGCACTGCACCACCGCCGTGATCAGGAACATCAACGACCAG

GTGCTGTTCGTGGACAAGAGGCAGCCCGTGTTTGAGGACATGACCGACATCGACCAGAGC

GCCAGCGAGCCCCAGACAAGGCTGATCATCTACATGTACAAGGACAGCGAGGTGAGGGGC

CTGGCCGTGACACTGAGCGTGAAGGACAGCAAGATGAGCACCCTGAGCTGCAAAAACAAG

ATCATCAGCTTCGAGGAGATGGACCCCCCCGAGAACATCGACGACATCCAGTCCGACCTG

ATCTTCTTCCAAAAGAGGGTGCCCGGCCACAACAAGATGGAGTTCGAGAGCAGCCTGTAT

GAGGGCCACTTCCTGGCCTGCCAGAAGGAGGATGATGCCTTCAAGCTGATCCTGAAGAAG

F2A

AAAGATGAGAACGGCGATAAGAGCGTGATGTTCACCCTGACCAACCTGCACCAGTCCGGC

AGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGACGTGGAGAGC

Mouse CCL19

AACCCTGGCCCATGCATGGCCCCCAGGGTGACCCCTCTGCTGGCCTTCAGCCTGCTGGTG

CTGTGGACCTTCCCTGCCCCTACCCTGGGAGGAGCCAATGACGCCGAGGACTGCTGCCTG

AGCGTGACCCAGAGACCCATCCCCGGCAACATCGTGAAGGCCTTCAGGTACCTGCTGAAC

GAGGACGGCTGCAGAGTGCCTGCCGTGGTGTTCACCACCCTGAGGGGCTACCAGCTGTGT

GCTCCCCCTGACCAGCCTTGGGTGGACAGGATCATCAGGAGGCTGAAGAAGAGCAGCGCC

AAGAACAAGGGCAACAGCACCAGGAGGAGCCCCGTGAGCTGAAAGCTTAAACCAGTTAAC

Stop codon

TGGAAAACGCGTAAAGTCGAC

Sal I

[図14]

配列番号14:IL-21_F2A_CCL19 DNA断片(DNA断片 #8)

F2A

GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC

Mouse IL-21

GTGGAGAGCAACCCTGGCCCTATGGAGAGGACCCTGGTGTGCCTGGTGGTGATTTTCCTG

GGCACCGTCGCCCATAAGAGCAGCCCCCAGGGACCCGACAGACTGCTGATCAGGCTGAGG

CACCTGATCGACATCGTGGAGCAGCTGAAGATCTACGAGAACGACCTGGACCCCGAGCTG

CTGAGCGCTCCTCAGGACGTGAAGGGACACTGCGAGCACGCCGCCTTCGCCTGTTTCCAG

AAGGCCAAGCTGAAGCCTTCCAACCCCGGCAACAACAAGACCTTCATCATCGACCTGGTG

GCCCAGCTGAGGAGAAGGCTGCCTGCCAGGAGGGGCGGAAAGAAGCAGAAGCACATCGCC

AAGTGCCCCAGCTGCGACTCCTACGAGAAGAGGACCCCCAAGGAGTTCCTGGAGAGGCTG

AAGTGGCTGCTGCAGAAGGTGTGCACACTGAACGCCTTCCTGAGCCTGCCTTGTTGCGTG

F2A

AGGGTGCCTCCCGTGCCTTCCGACAGCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGAC

Mouse CCL19

CTGCTGAAGCTGGCCGGAGACGTGGAGAGCAACCCTGGCCCATGCATGGCCCCCAGGGTG

ACCCCTCTGCTGGCCTTCAGCCTGCTGGTGCTGTGGACCTTCCCTGCCCCTACCCTGGGA

GGAGCCAATGACGCCGAGGACTGCTGCCTGAGCGTGACCCAGAGACCCATCCCCGGCAAC

ATCGTGAAGGCCTTCAGGTACCTGCTGAACGAGGACGGCTGCAGAGTGCCTGCCGTGGTG

TTCACCACCCTGAGGGGCTACCAGCTGTGTGCTCCCCCTGACCAGCCTTGGGTGGACAGG

ATCATCAGGAGGCTGAAGAAGAGCAGCGCCAAGAACAAGGGCAACAGCACCAGGAGGAGC

CCCGTGAGCTGAAAGCTTAAACCAGTTAACTGGAAAACGCGTAAAGTCGAC

Stop codon

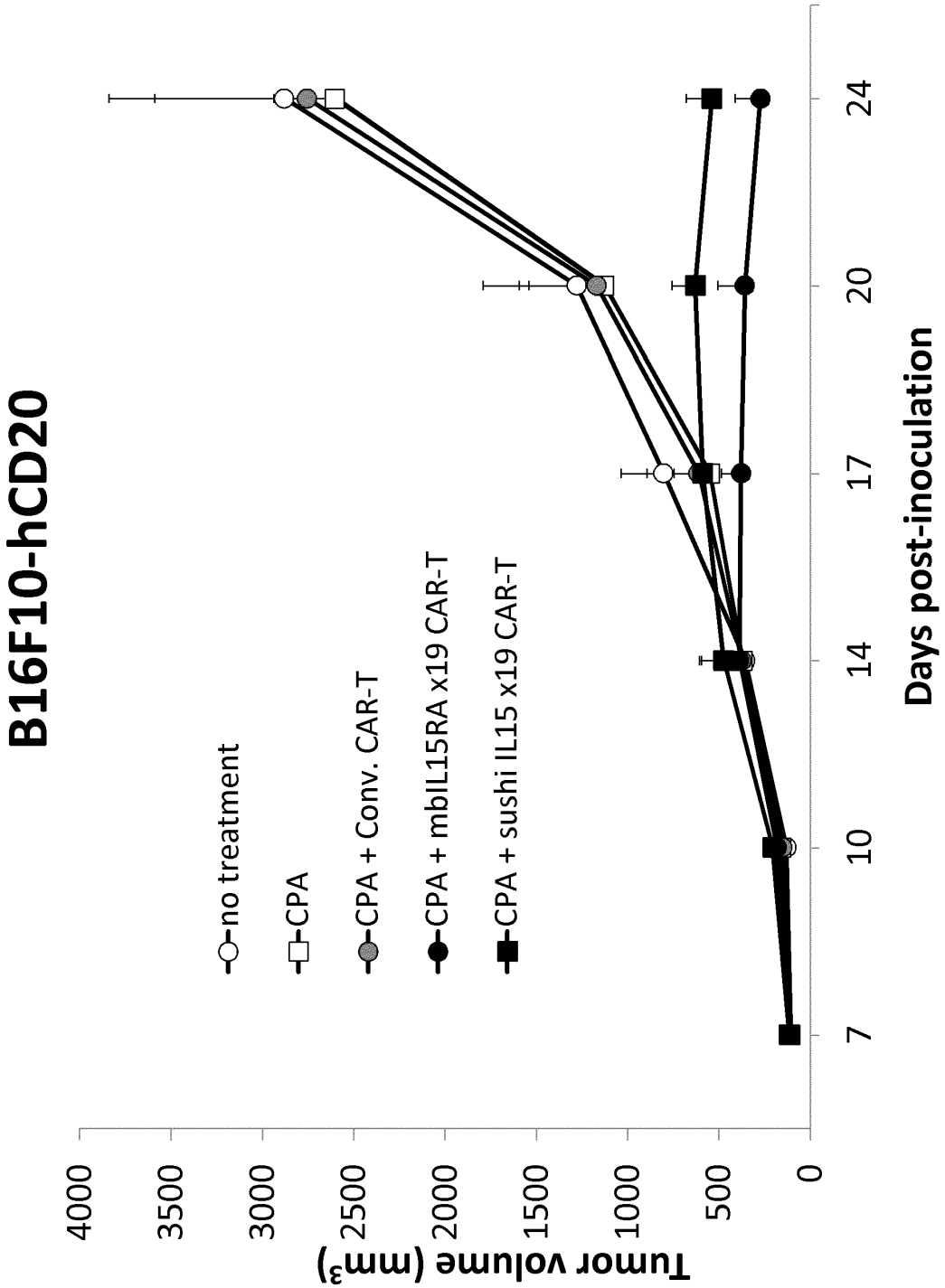
Sal I

[図15]

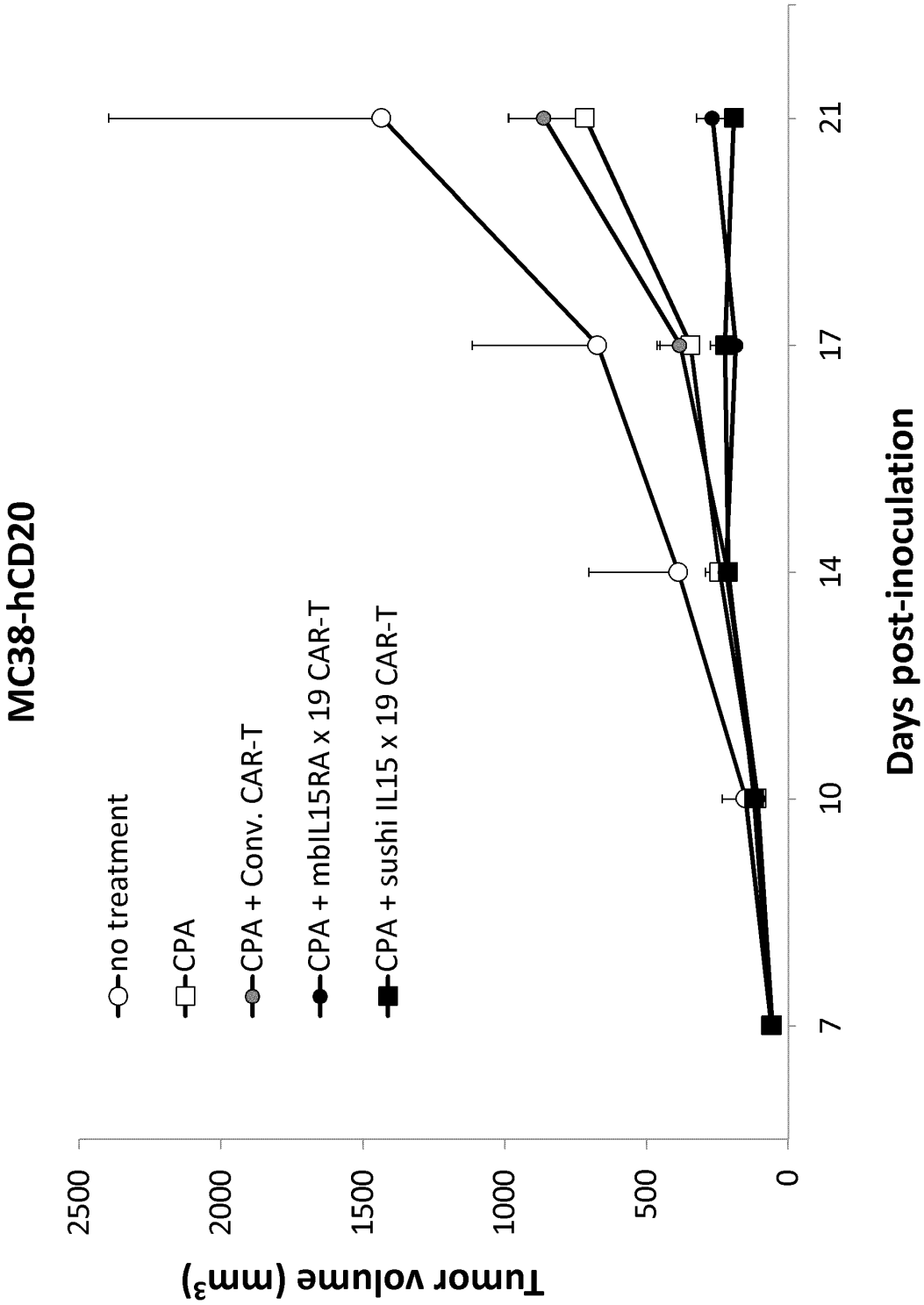
配列番号16：$_{sc}$IL27_F2A_CCL19 DNA断片（DNA断片 #9）

F2A
GAATTCGGCAGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGAC
Mouse EBI3
GTGGAGAGCAACCCTGGCCCTATGAGCAAGCTCCTCTTTCTGTCCCTGGCCCTGTGGGCC
AGCAGATCCCCTGGCTACACAGAAACAGCCCTGGTGGCCCTGTCCCAACCCAGAGTGCAG
TGTCACGCTTCCAGGTACCCCGTGGCTGTGGACTGCAGCTGGACCCCTCTGCAAGCCCCT
AATAGCACCAGGAGCACCTCCTTCATCGCCACCTACAGACTGGGAGTGGCCACCCAGCAG
CAGAGCCAGCCTTGCCTGCAGAGGTCCCCTCAGGCCTCCAGATGCACAATCCCTGATGTG
CACCTGTTTAGCACCGTGCCCTACATGCTGAACGTGACAGCCGTGCACCCTGGAGGAGCC
AGCAGCTCCCTCCTGGCCTTCGTCGCCGAGAGAATCATCAAGCCTGACCCCCCTGAGGGC
GTGAGACTGAGAACAGCTGGCCAGAGACTGCAGGTGCTGTGGCACCCTCCCGCCTCCTGG
CCTTTCCCCGATATCTTCTCCCTGAAGTACAGACTGAGGTACAGGAGAAGAGGCGCTAGC
CACTTCAGACAGGTGGGCCCCATTGAGGCTACCACCTTTACCCTCAGGAATAGCAAGCCC
CATGCTAAGTACTGTATCCAGGTGTCCGCCCAGGATCTGACAGACTACGGCAAGCCCTCC
18aa linker
GACTGGAGCCTGCCTGGACAAGTGGAAAGCGCTCCCCACAAGCCTGGAAGCACATCCGGA
p28
AGCGGCAAACCCGGCAGCGGAGAGGGATCCACCAAAGGCTTCCCTACCGACCCCCTGTCT
TTACAAGAACTGAGAAGGGAGTTCACCGTGAGCCTGTATCTGGCCAGGAAGCTCCTCAGC
GAGGTGCAGGGCTATGTGCACAGCTTCGCCGAAAGCAGGCTGCCCGGCGTGAACCTGGAT
CTGCTGCCCCTGGGCTACCACCTGCCTAACGTGTCCCTGACCTTCCAGGCTTGGCATCAC
CTGTCCGACTCCGAGAGGCTCTGCTTTCTGGCCACCACCCTGAGGCCCTTCCCTGCCATG
CTGGGAGGACTGGGCACCCAGGGAACCTGGACCAGCAGCGAGAGGGAACAGCTGTGGGCC
ATGAGACTGGACCTGAGGGATCTGCACAGACACCTGAGGTTCCAGGTGCTGGCCGCCGGC
TTCAAGTGTAGCAAAGAGGAGGAGGACAAAGAAGAAGAAGAAGAGGAAGAAGAGGAAGAG
AAGAAACTCCCCCTGGGAGCCCTGGGAGGCCCTAATCAGGTGTCCTCCCAGGTGTCCTGG
CCTCAGCTGCTGTATACCTACCAGCTGCTGCACAGCCTGGAGCTGGTGCTGTCCAGGGCT
F2A
GTGAGGGACCTGCTCCTGCTGAGCCTGCCTAGAAGGCCTGGCAGCGCTTGGGACTCCGGC
AGCGGCGTGAAGCAGACCCTGAACTTCGACCTGCTGAAGCTGGCCGGAGACGTGGAGAGC
Mouse CCL19
AACCCTGGCCCATGCATGGCCCCCAGGGTGACCCCTCTGCTGGCCTTCAGCCTGCTGGTG
CTGTGGACCTTCCCTGCCCCTACCCTGGGAGGAGCCAATGACGCCGAGGACTGCTGCCTG
AGCGTGACCCAGAGACCCATCCCCGGCAACATCGTGAAGGCCTTCAGGTACCTGCTGAAC
GAGGACGGCTGCAGAGTGCCTGCCGTGGTGTTCACCACCCTGAGGGGCTACCAGCTGTGT
GCTCCCCCTGACCAGCCTTGGGTGGACAGGATCATCAGGAGGCTGAAGAAGAGCAGCGCC
AAGAACAAGGGCAACAGCACCAGGAGGAGCCCCGTGAGCTGAAAGCTTAAACCAGTTAAC
Stop codon
TGGAAAACGCGTAAAGTCGAC
Sal I

[図16]


B16F10-hCD20
no treatment
CPA
CPA + Conv. CAR-T
CPA + mbIL15RA x19 CAR-T
CPA + sushi IL15 x19 CAR-T
Tumor volume (mm3)
0
500
1000
1500
2000
2500
3000
3500
4000
Days post-inoculation
7
10
14
17
20
24

[図17]


MC38-hCD20
no treatment
CPA
CPA + Conv. CAR-T
CPA + mbIL15RA x 19 CAR-T
CPA + sushi IL15 x 19 CAR-T
Tumor volume (mm3)
2500
2000
1500
1000
500
0
7
10
14
17
21
Days post-inoculation