Caushi JX and Zhang J *et al*,

Distinct transcriptional programs characterize neoantigen-specific TIL in lung cancers treated with anti-PD-1

Supplementary Data File 5

Table of contents

Supplementary Data 5.1…….. Page 2

MANA-specific TCRs identified in MPR and non-MPR patients using the MANAFEST assays

Supplementary Data 5.2……..… Page 9

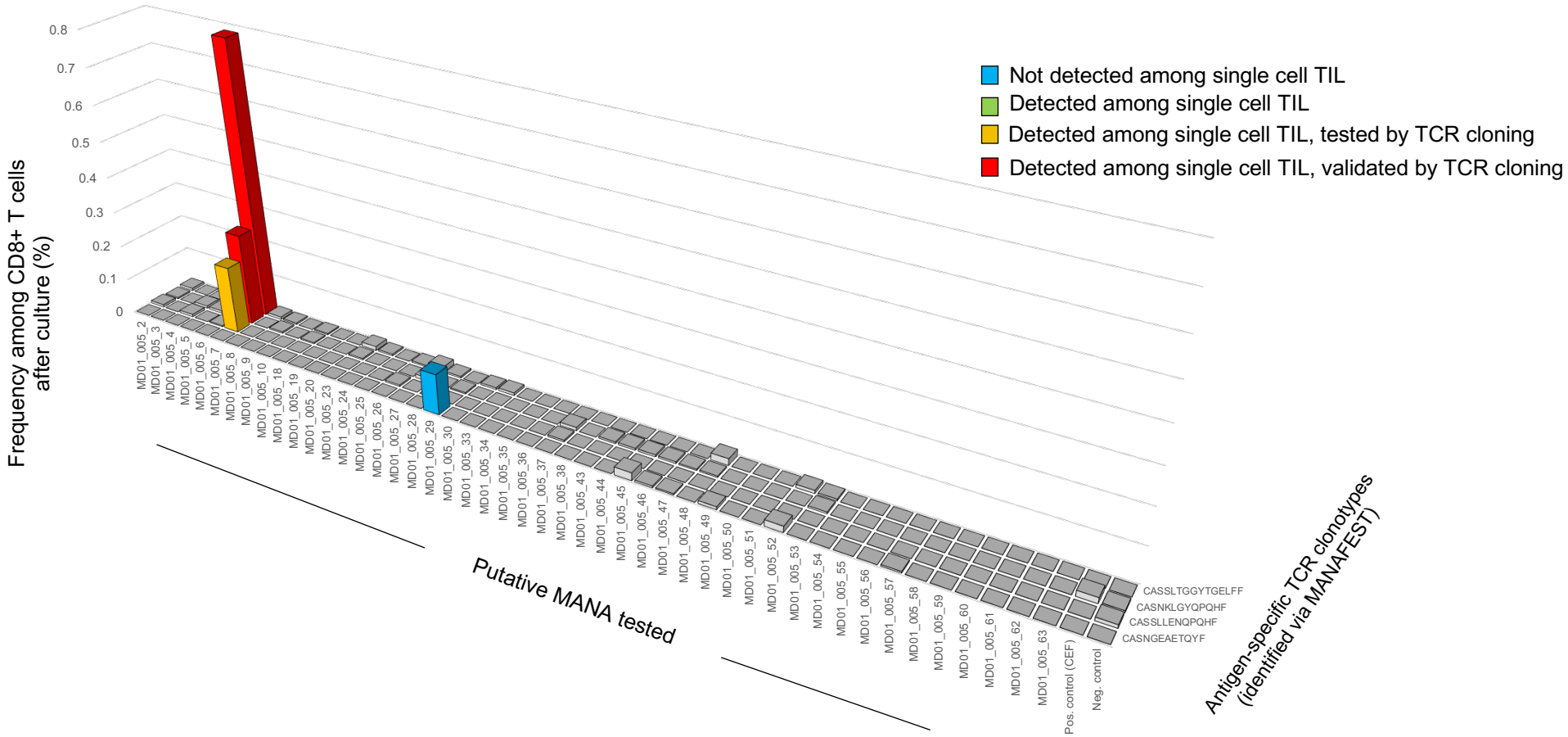
Influenza-specific TCRs identified using the ViraFEST assay

Supplementary Data 5.3……. Page 12

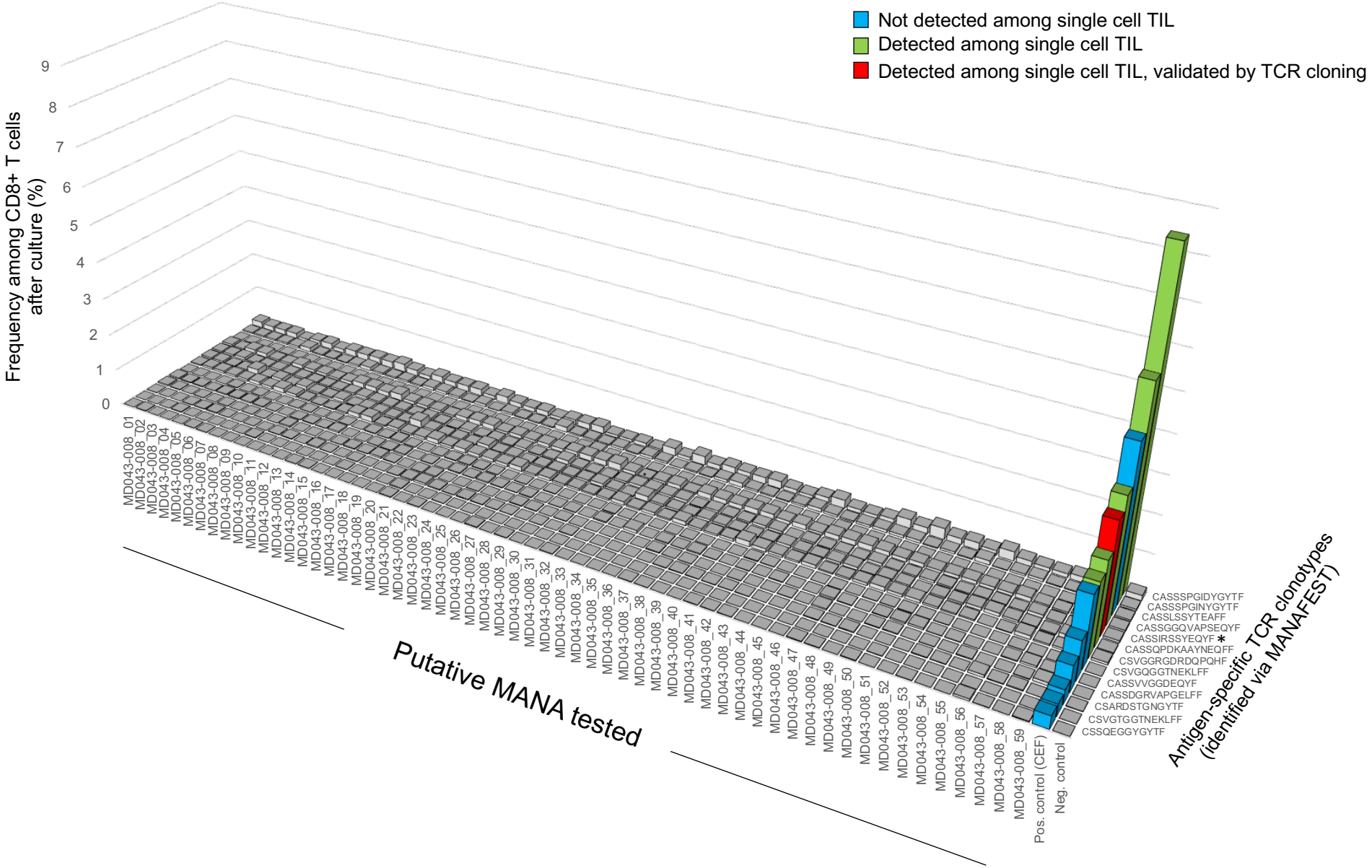
The frequency of each MANA-specific TCR Vβ clonotype in each condition tested in the MANAFEST assay visualized by individual dot plots

Supplementary Data 5.1 MANA-specific TCRs identified in MPR and non-MPR patients using the MANAFEST assays. Antigen-specific responses identified using the MANAFEST assay are shown for MPRs MD01-005 (re-analyzed results are shown below; original results previously reported for this patient in Forde PM et al, N Engl J Med, 2018), MD043-008, MD043-003, and NY016-025. Antigen-specific responses identified using the MANAFEST assay are shown for non-MPRs MD043-011, NY016-007, and NY016-014. Each antigen-specific clonotypic expansion is color coded to indicate if the clone was not detected in the single cell data (blue), detected in the single cell data but not tested via TCR cloning (green), detected in the single cell data but did not validate with TCR cloning (orange), or detected in the single cell data and validated with TCR cloning (red). Data are shown as the percent of MANAFEST+ clonotypes among CD8+ T cells after 10 day culture. Neither MANA-specific nor CEF-specific T cell responses were detected in non-MPR patient, MD01-019. *Clones determined to recognize CEF were queried against a database of TCRs with known specificity (vdjdb, Supplementary Table 8). Several clonotypes were previously been reported to recognize common viral epitopes used in the CEF pool. Two of these clonotypes reported to recognize the influenza A matrix protein HLA A*02:01-restricted GILGFVFTL epitope. The full TCR corresponding to these clonotypes were cloned into the Jurkat transfer system and were validated to recognize the reported influenza antigen. Composite 3D bar plot for MD01-004 is shown in **Extended Data Fig. 2**.

Patient MD01-005



Patient MD043-008



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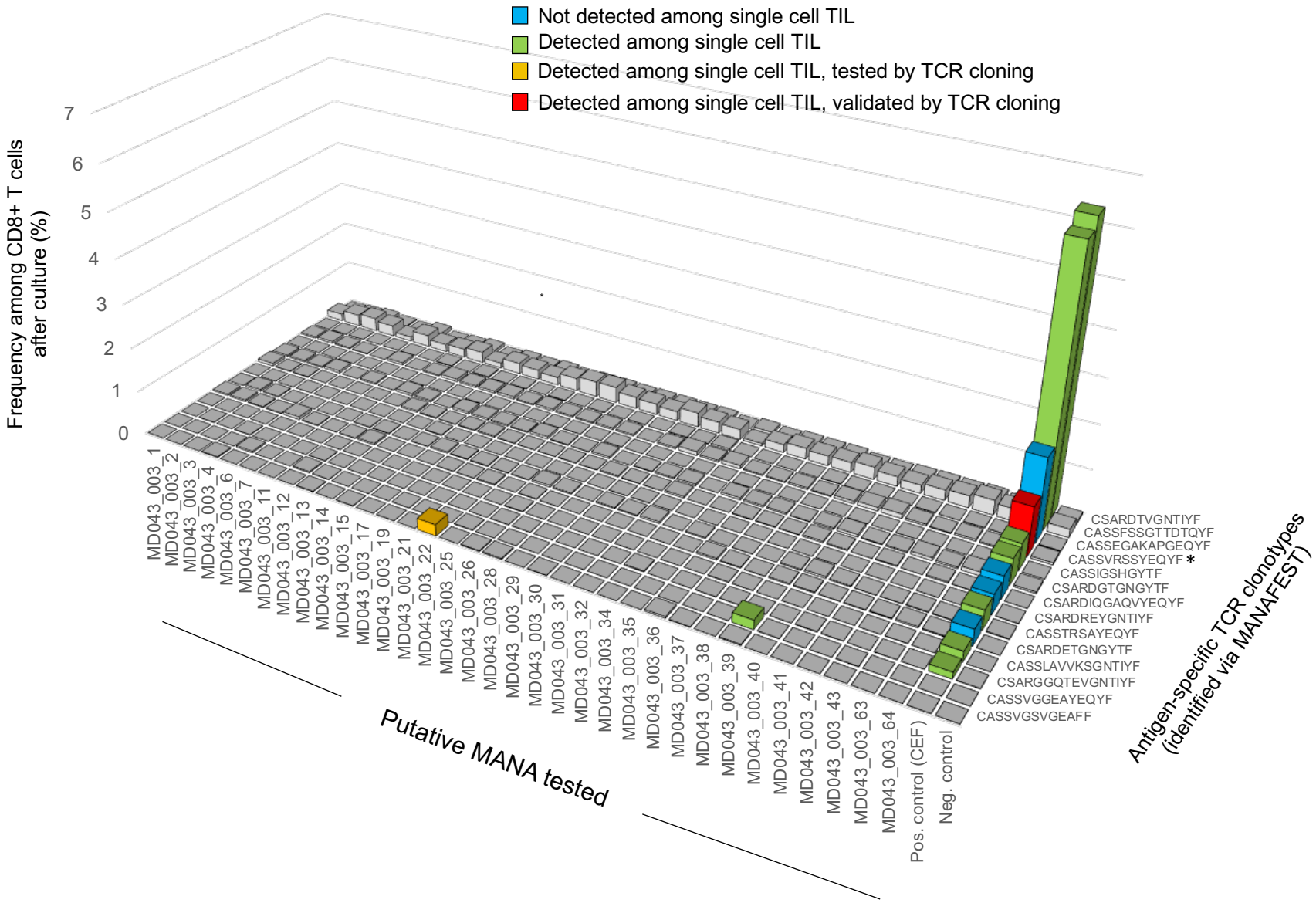
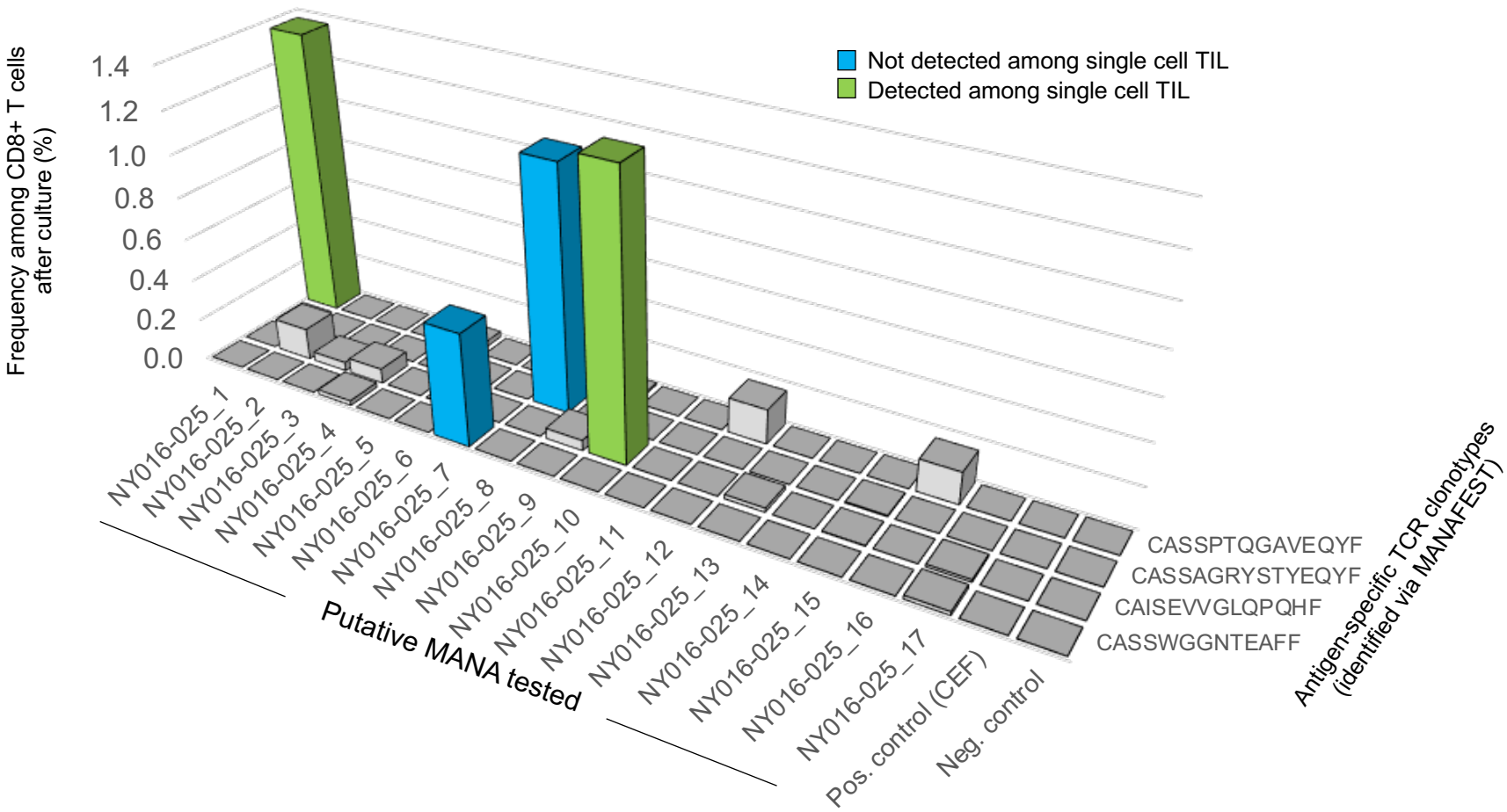
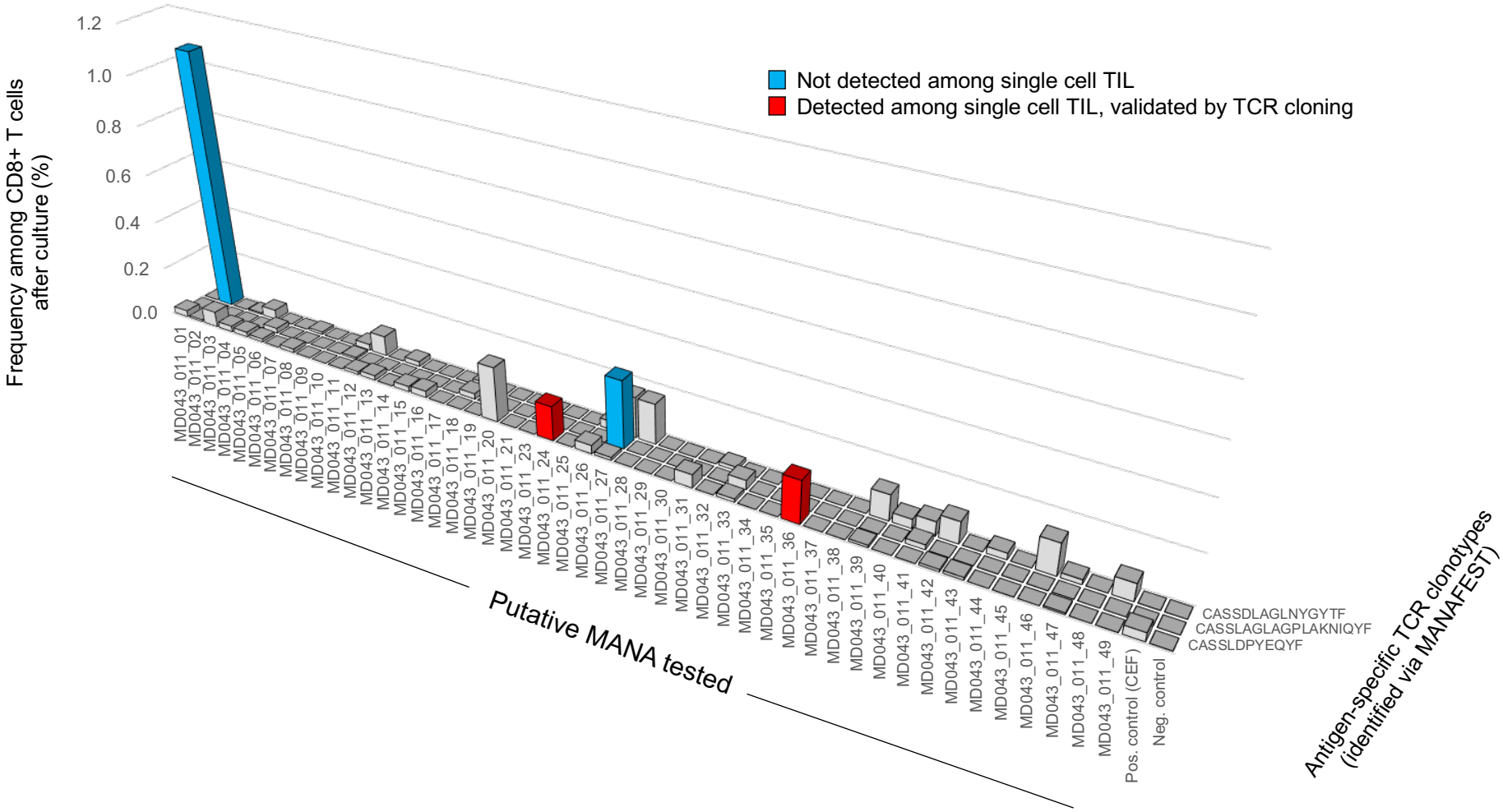


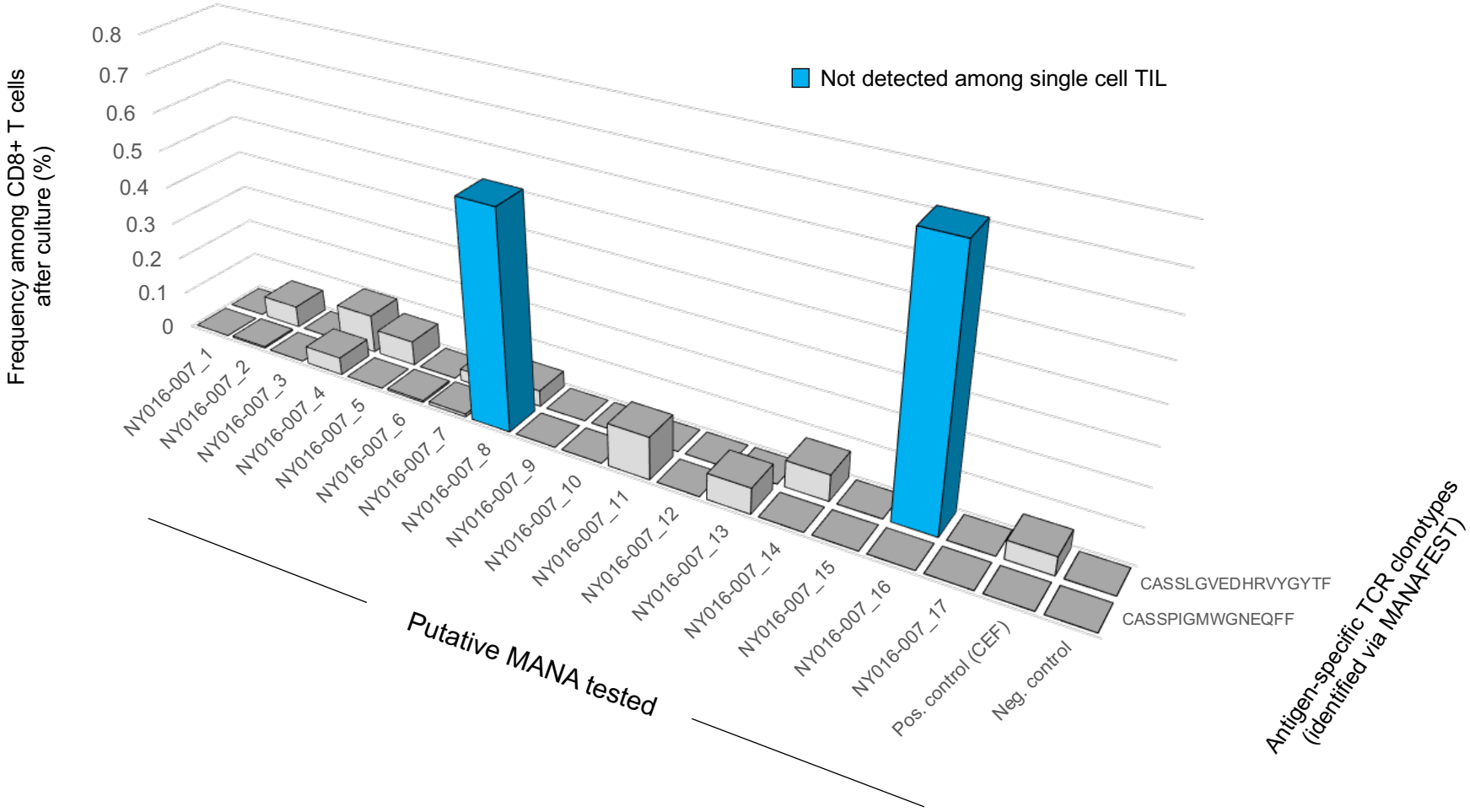
Patient NY016-025



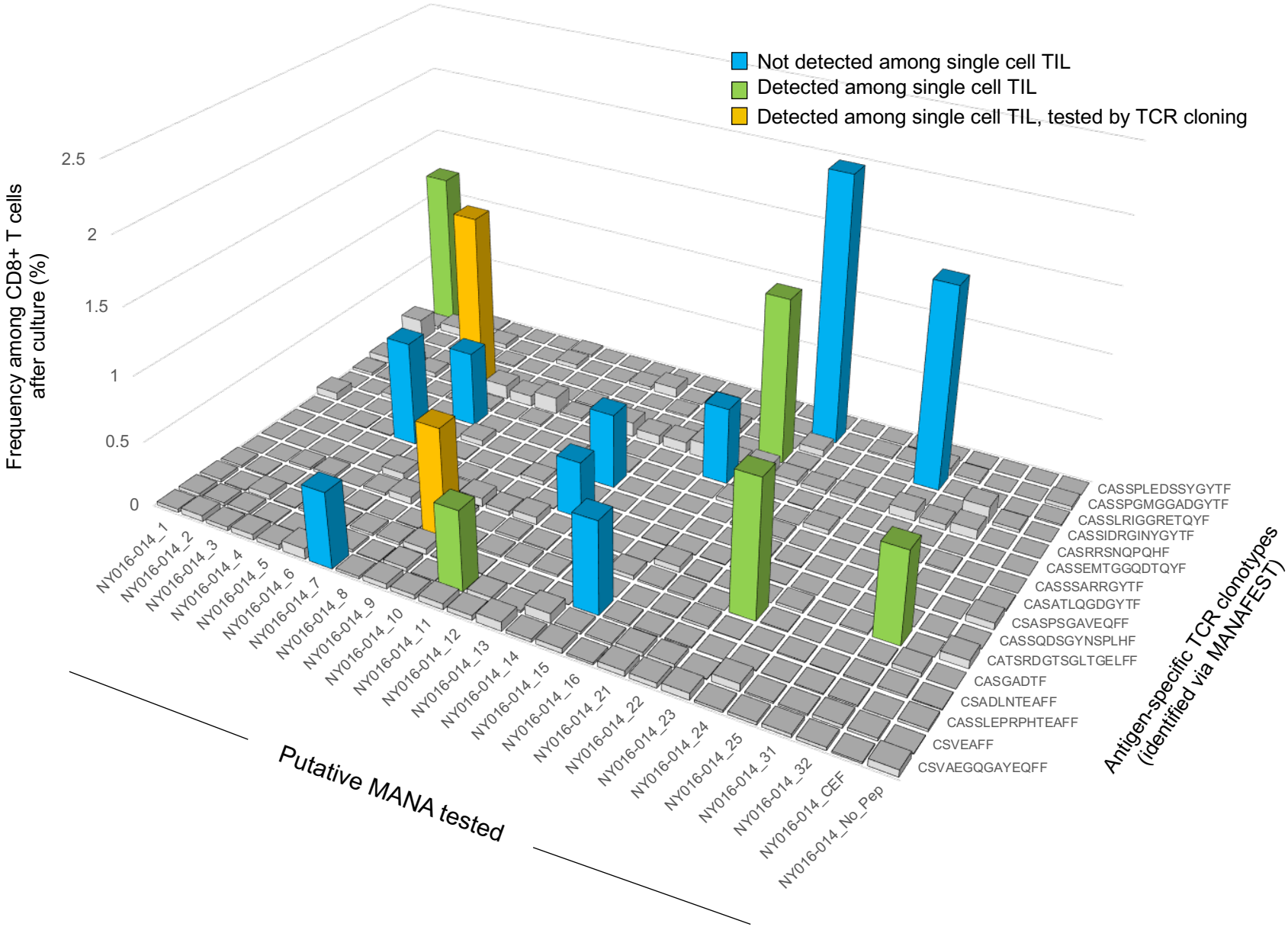
Patient MD043-011



Patient NY016-007

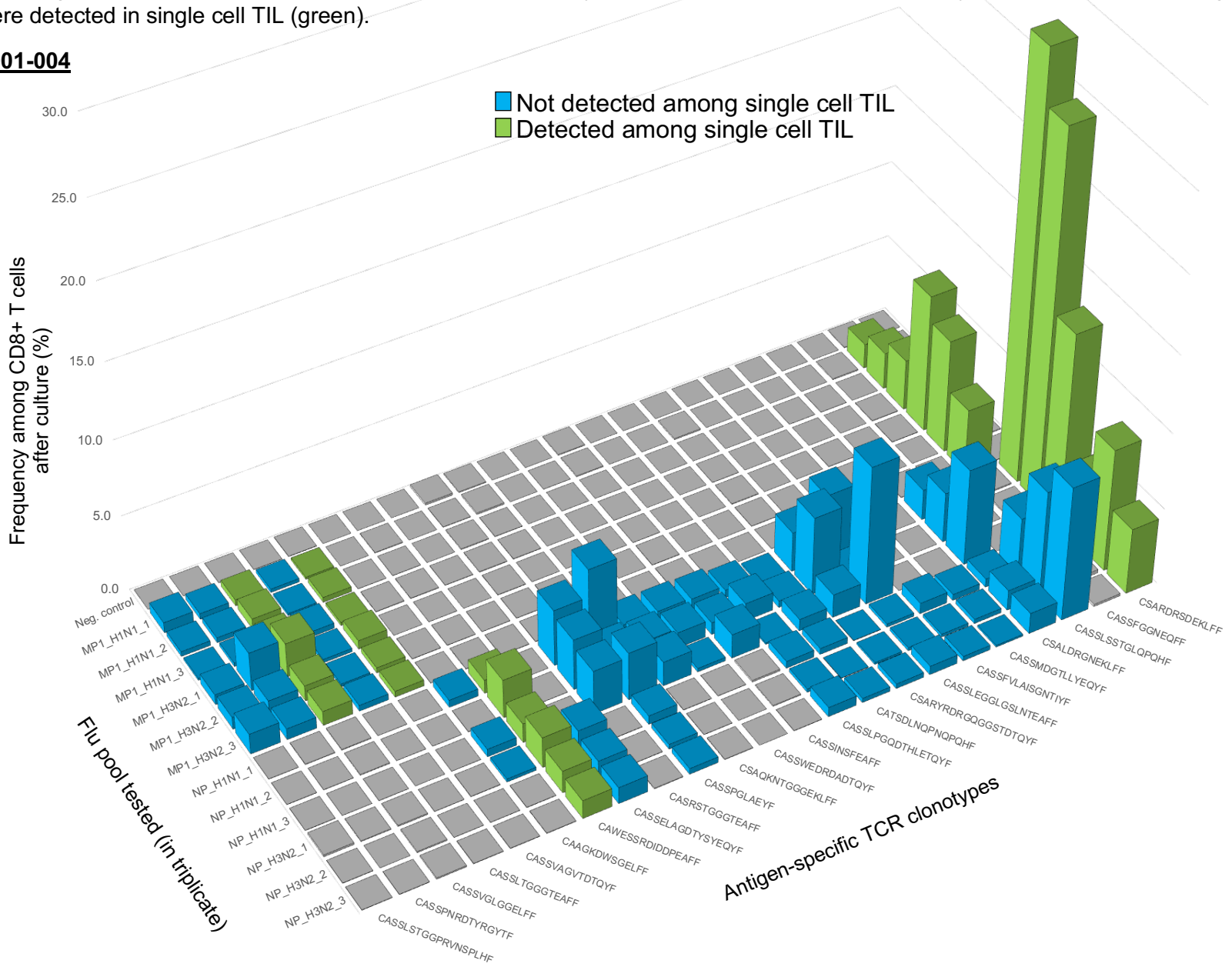


Patient NY016-014

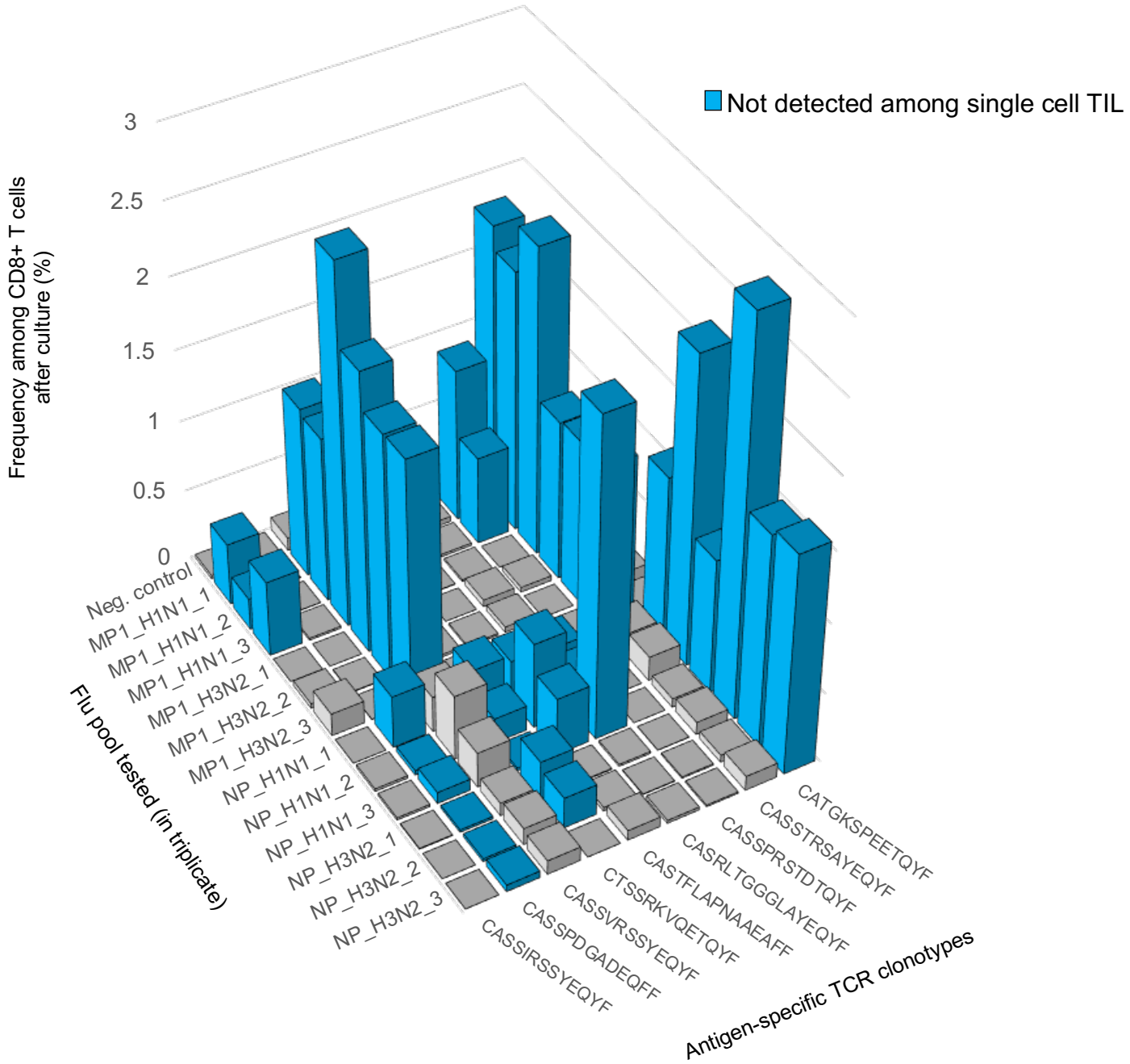


Supplementary Data 5.2. **Influenza-specific TCRs identified using the ViraFEST assay.** The ViraFEST assay was performed on non-MPR patients MD01-004 and MD043-011, and MPR patient MD01-005 to identify influenza-specific TCRβ clones. Influenza A pools consisting of overlapping peptides from the matrix and nucleocapsid proteins of H1N1 and H3N2, or an HIV-1 Gag pool as a negative control, were used to stimulate peripheral blood T cells in vitro for 10 days. Each flu peptide pool was tested in triplicate. Data are shown as the frequency of ViraFEST+ clonotypes among total $CD8^+$ T cells for each culture condition. Clonotypes are color coded to indicate if they were not detected in single cell TIL (blue) or were detected in single cell TIL (green).

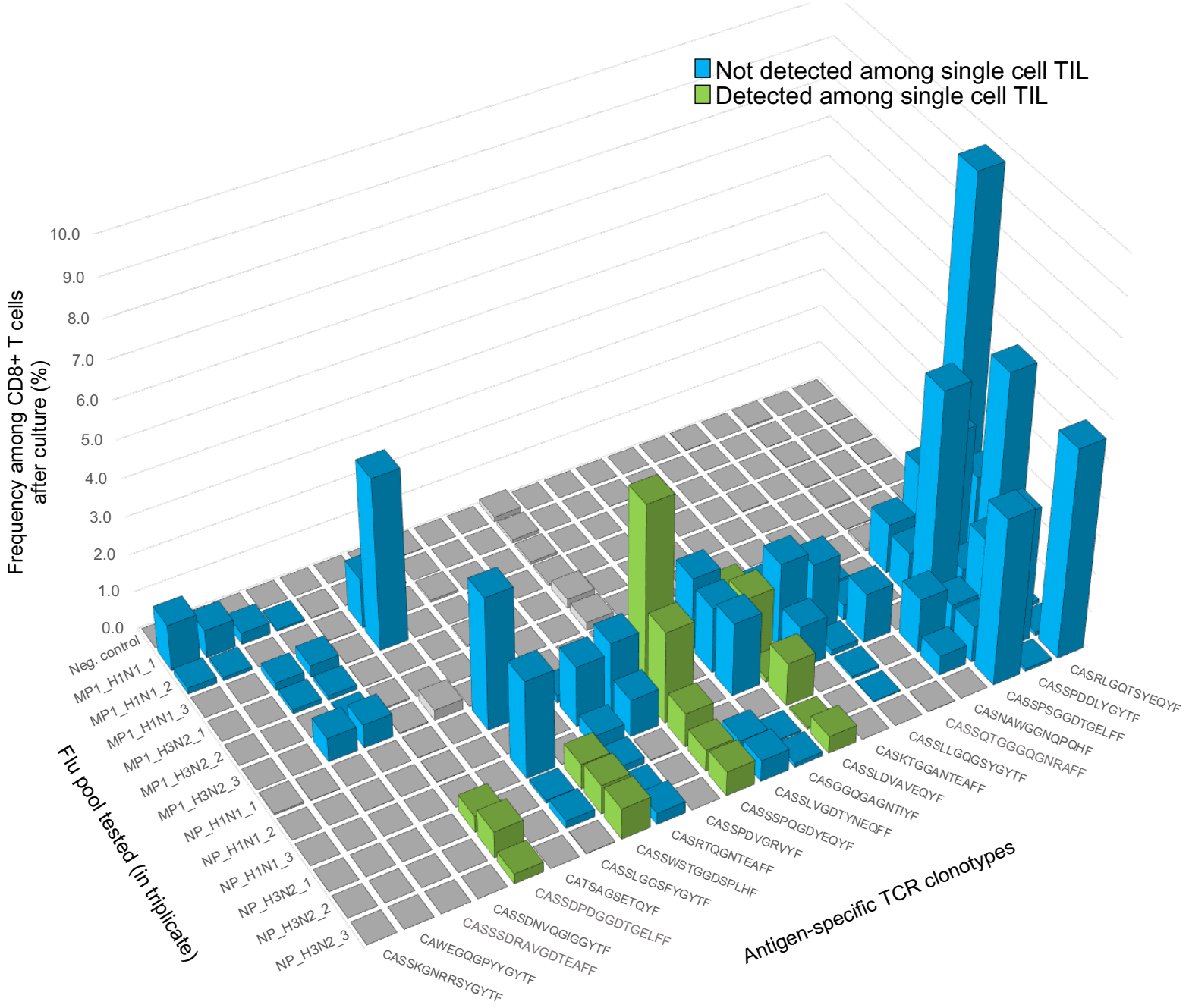
Patient MD01-004



Patient MD043-011

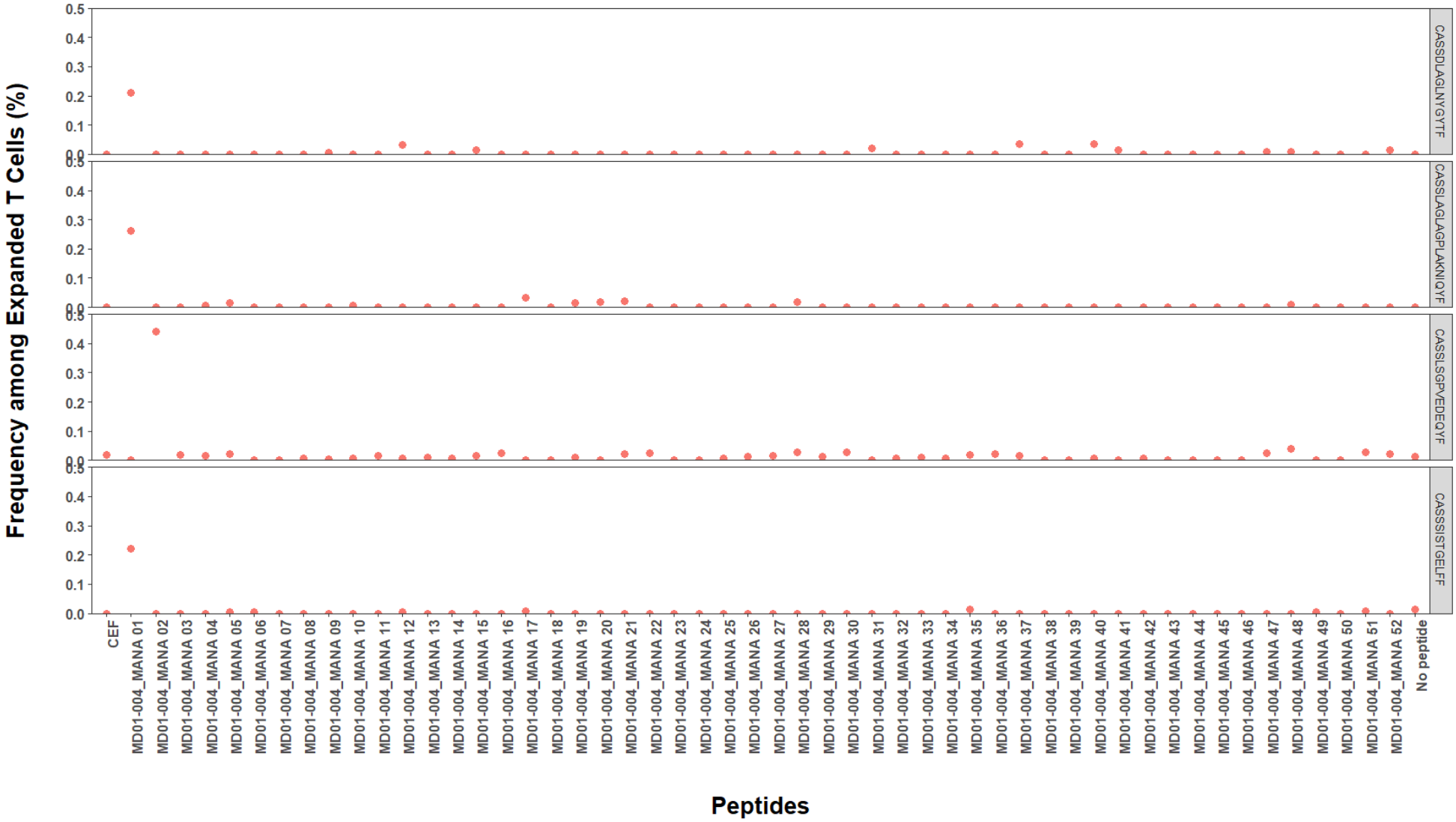


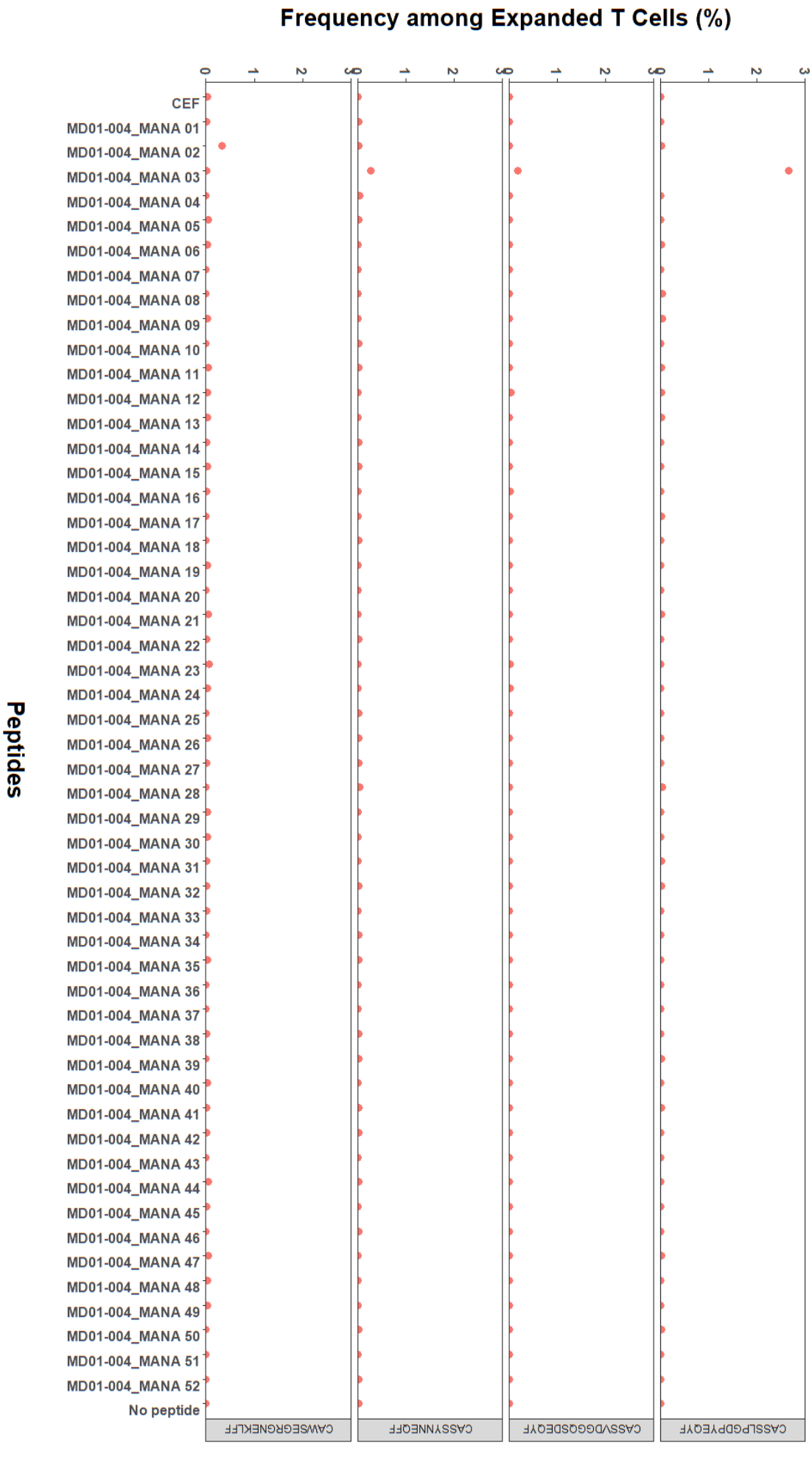
Patient MD01-005



Supplementary Data 5.3 The frequency of each MANA-specific TCR Vβ clonotype in each condition tested in the MANAFEST assay visualized by individual dot plots. Peripheral blood T cells were tested for reactivity to putative MANA using the MANAFEST assay. A graph is shown for each MANA- and CEF-specific TCR Vβ clonotype (right y-axis) detected in each patient. The frequency of each clonotype is shown as the percent among all T cells detected by TCRseq after culture (left y-axis) in each peptide-stimulated condition, the CEF positive control well, and the "no peptide" negative control well (x-axis).

Patient MD01-004




Frequency among Expanded T Cells (%)
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1
2
3
Peptides
CEF
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MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
MD01-004_MANA 07
MD01-004_MANA 08
MD01-004_MANA 09
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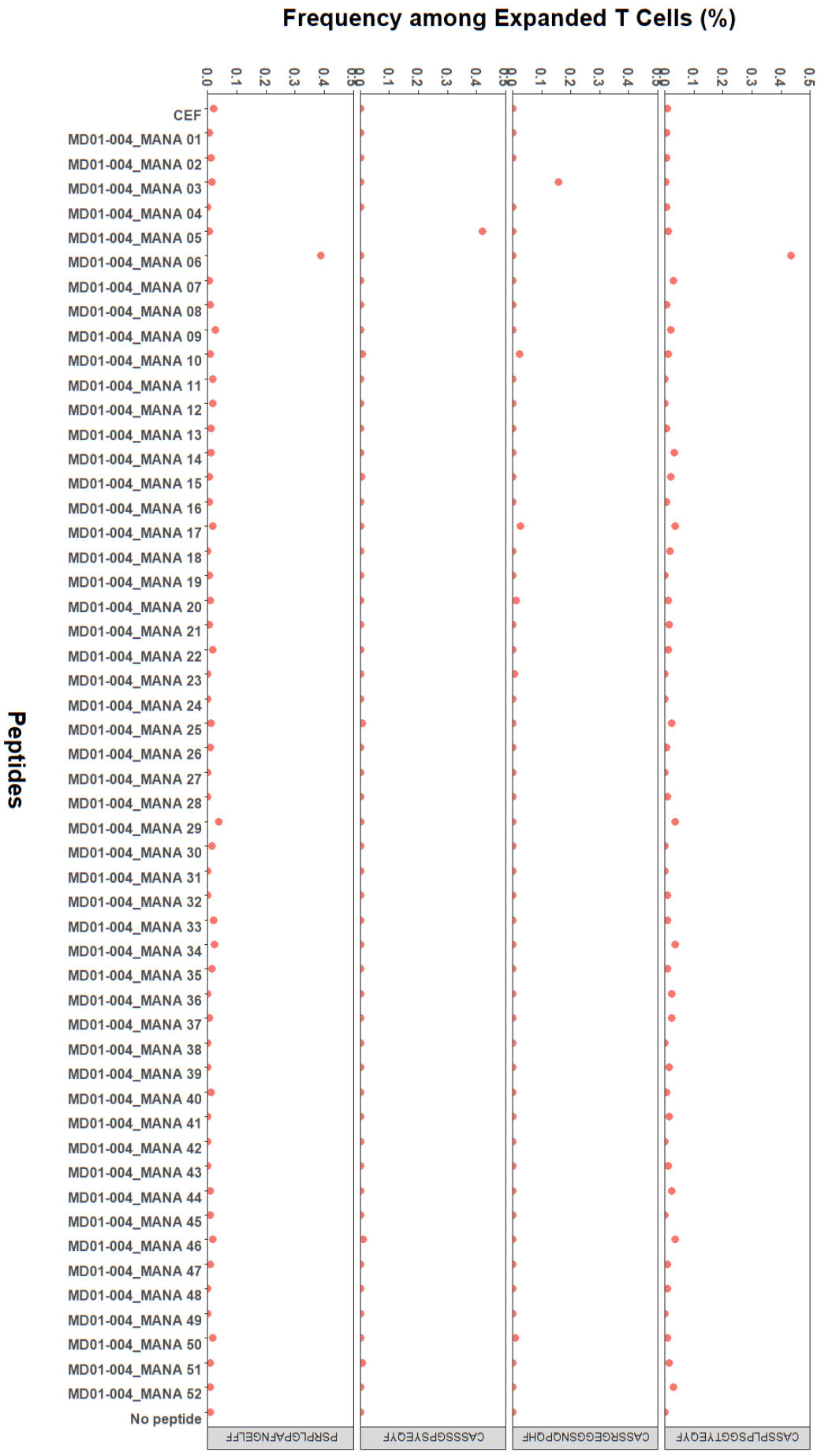
CASSLPGDPYEQYF


Frequency among Expanded T Cells (%)
Peptides
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MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
MD01-004_MANA 07
MD01-004_MANA 08
MD01-004_MANA 09
MD01-004_MANA 10
MD01-004_MANA 11
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MD01-004_MANA 51
MD01-004_MANA 52
No peptide
0.0
0.1
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0.5
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CASSRGEGGSNQPQHF
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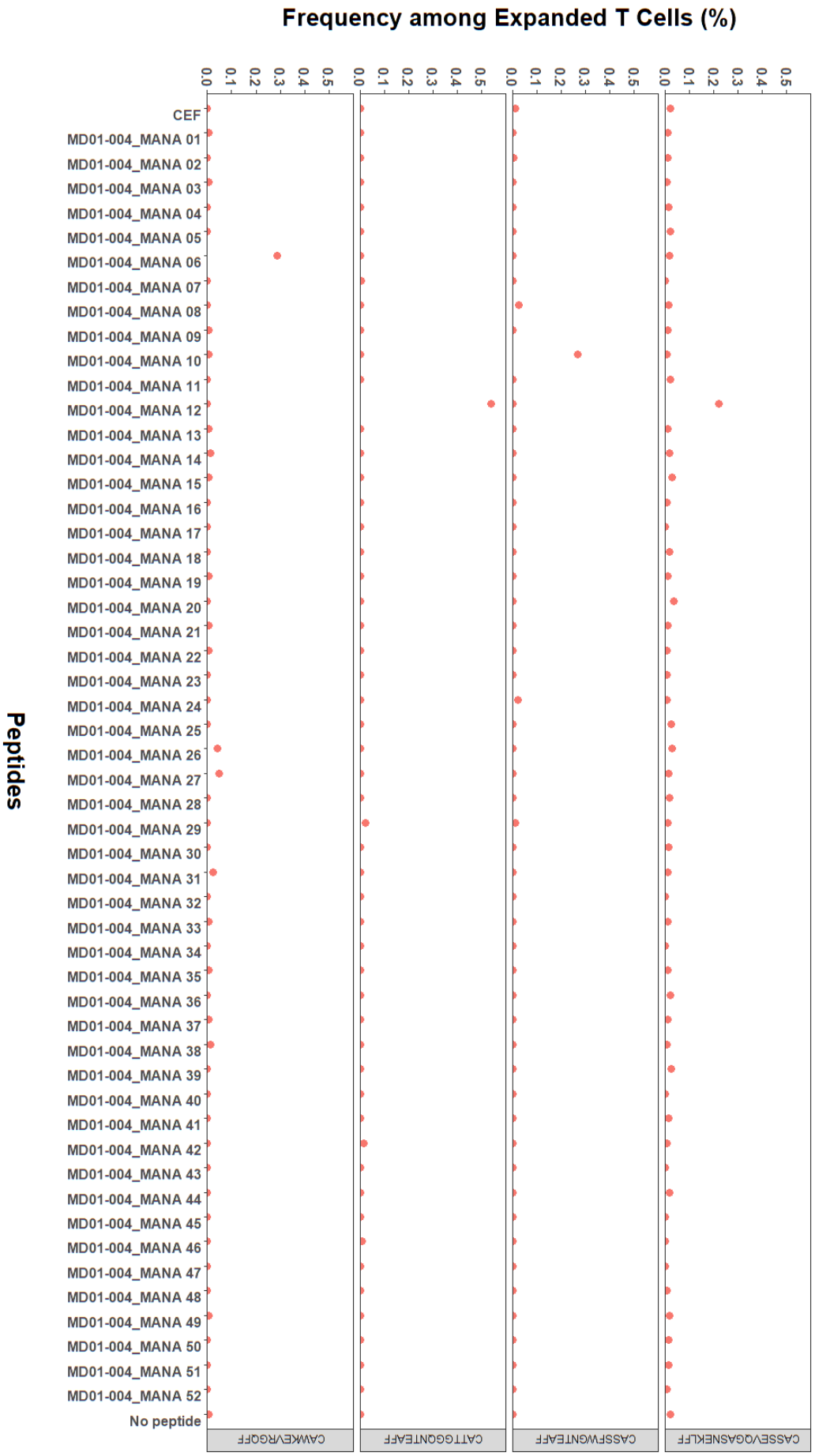
Frequency among Expanded T Cells (%)
0.0
0.1
0.2
0.3
0.4
0.5
Peptides
CEF
MD01-004_MANA 01
MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
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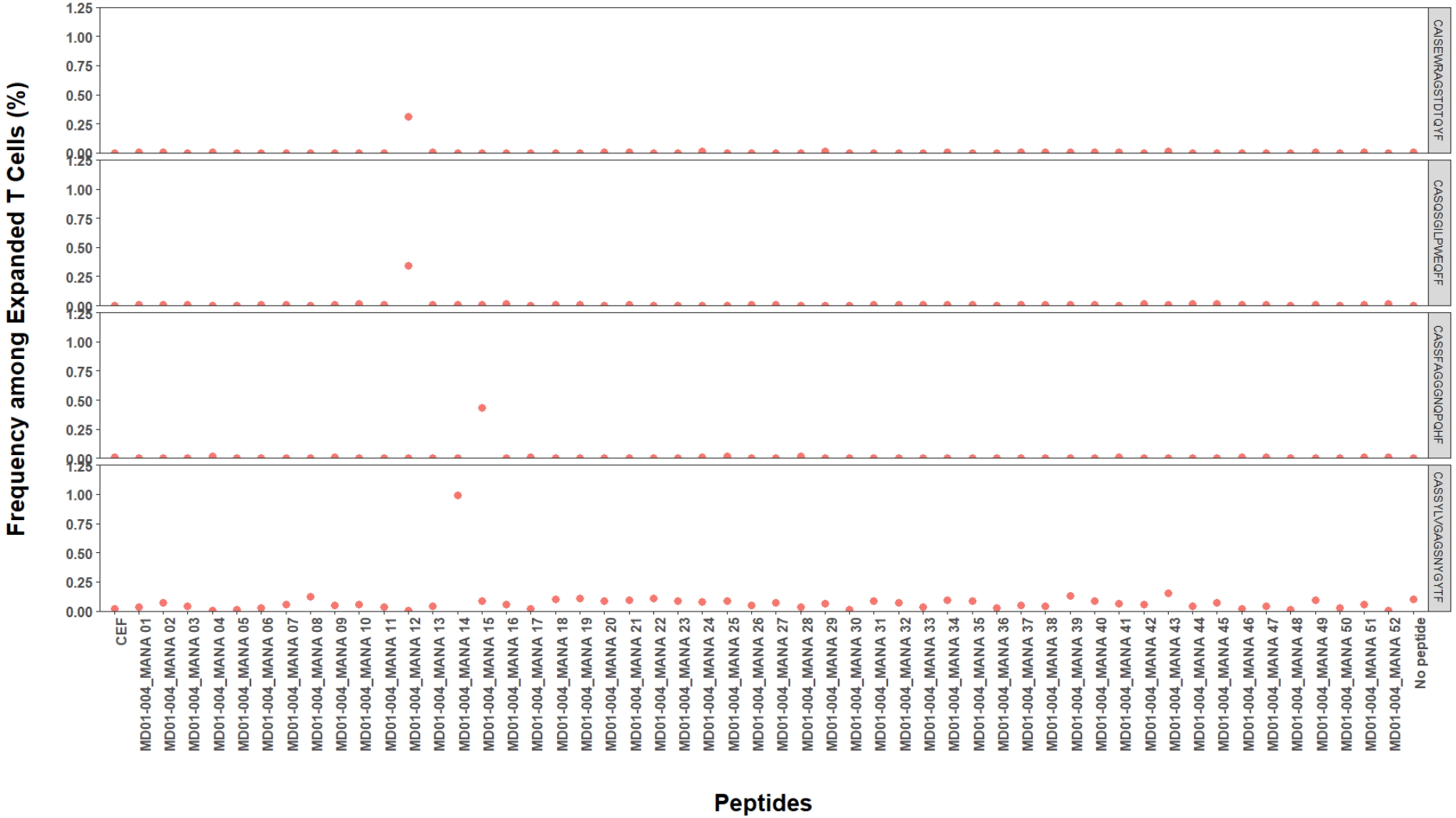
Frequency among Expanded T Cells (%)
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0.75
0.50
0.25
0.00
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CASSFAGGGNQPQHF
CASSYLVGAGSNYGYTF
CEF
MD01-004_MANA 01
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MD01-004_MANA 52
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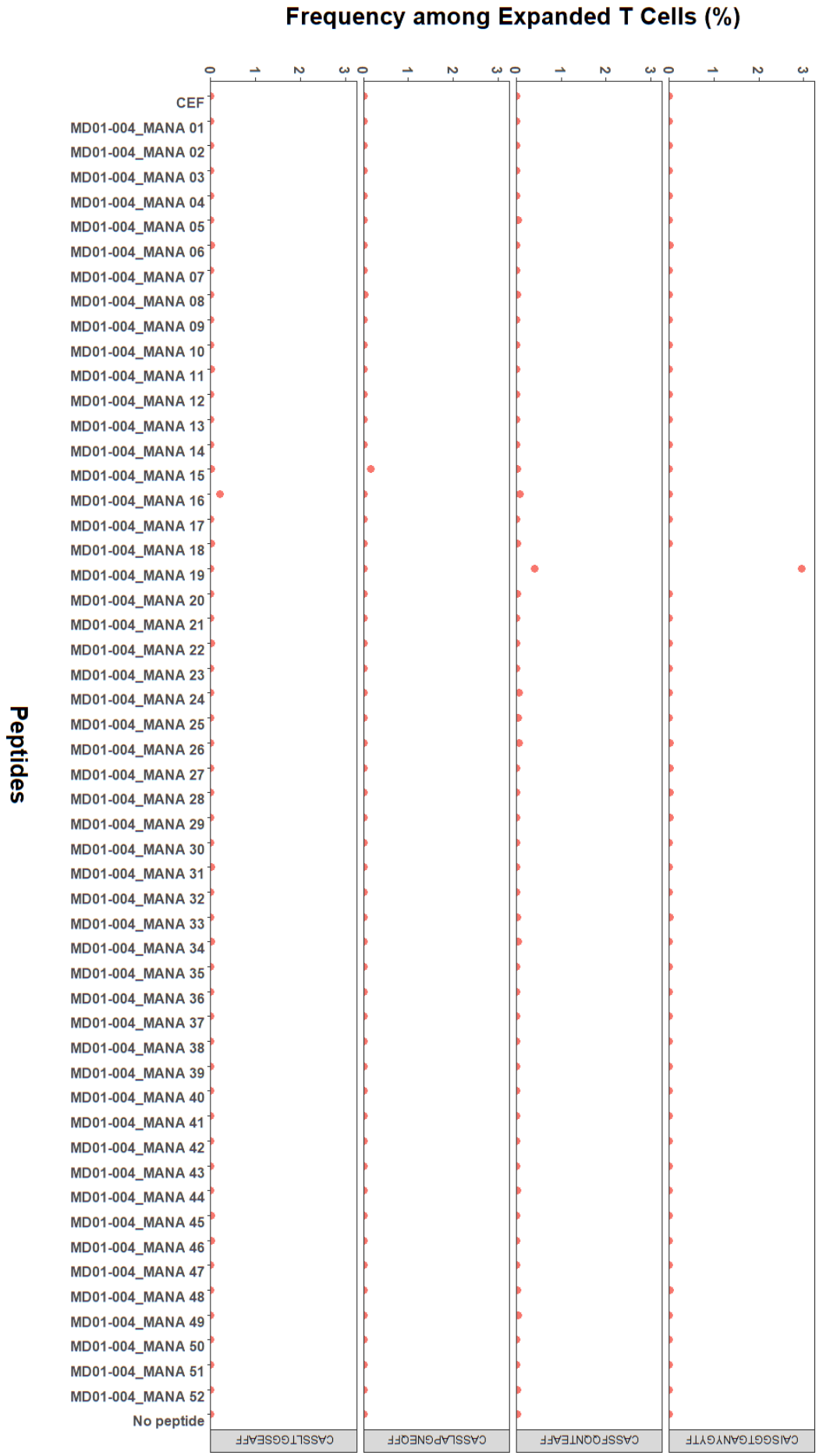
Peptides


Frequency among Expanded T Cells (%)
0
1
2
3
Peptides
CEF
MD01-004_MANA 01
MD01-004_MANA 02
MD01-004_MANA 03
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MD01-004_MANA 05
MD01-004_MANA 06
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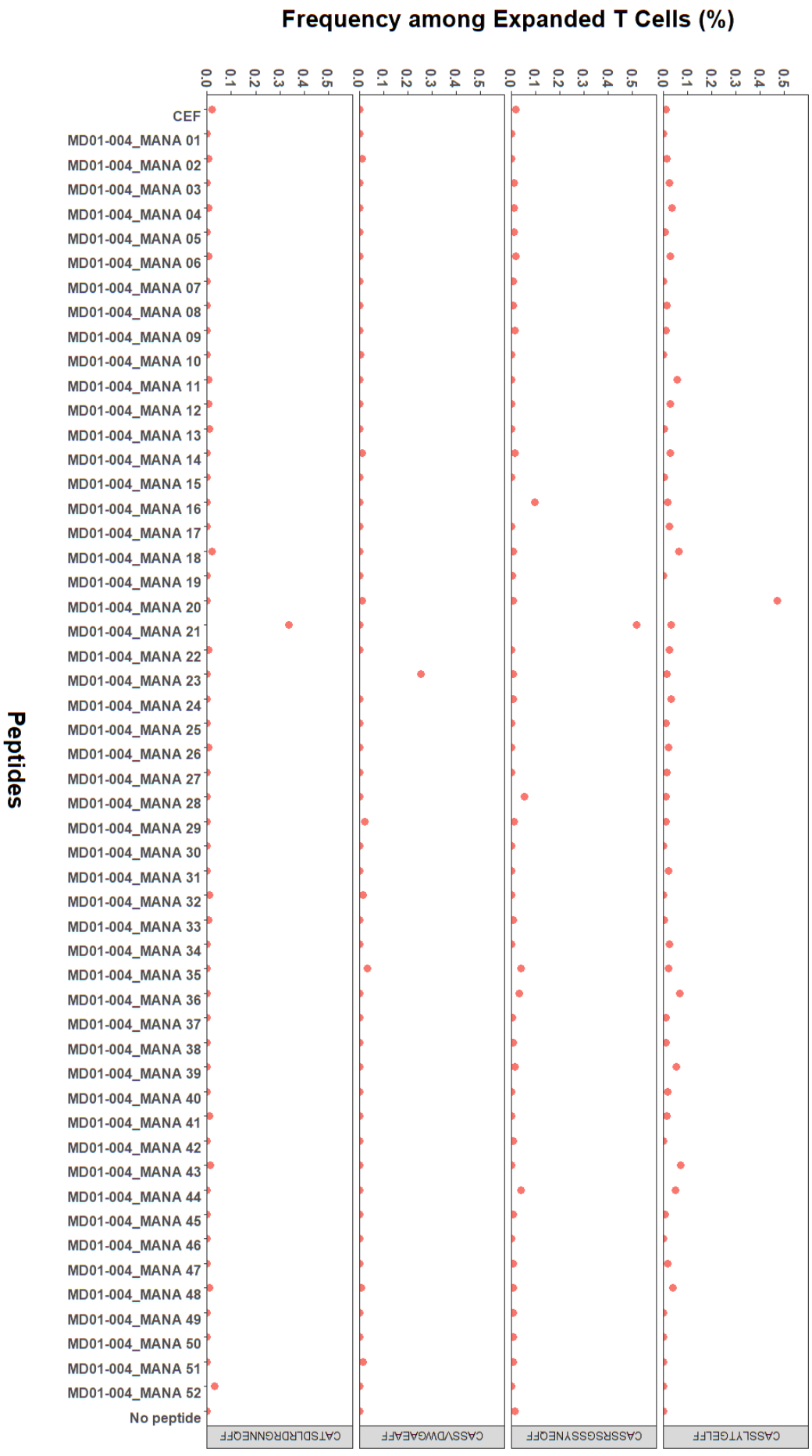
Frequency among Expanded T Cells (%)
0.0
0.1
0.2
0.3
0.4
0.5
Peptides
CEF
MD01-004_MANA 01
MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
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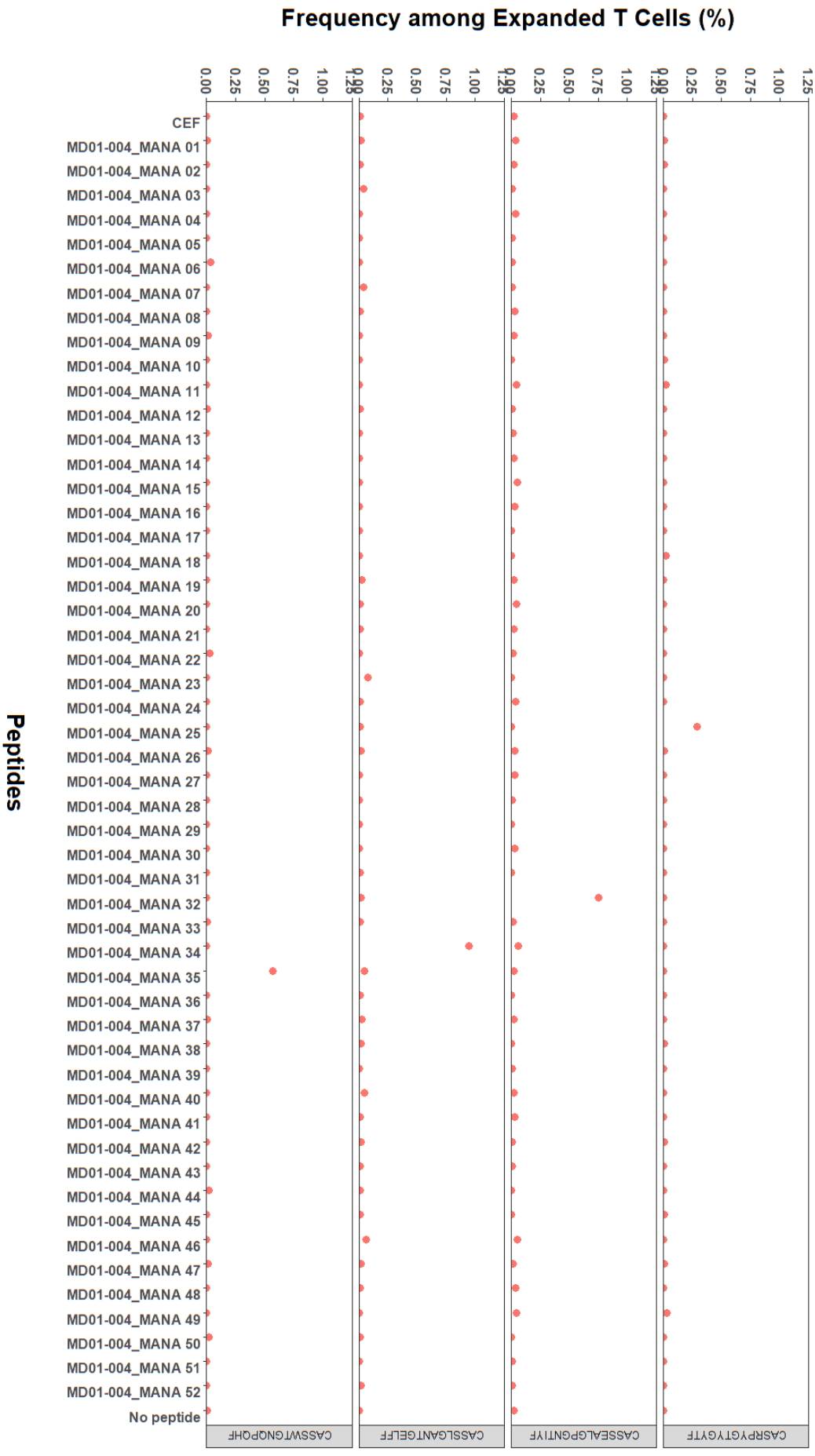
CASSLYTGELFF


Frequency among Expanded T Cells (%)
0.00
0.25
0.50
0.75
1.00
1.25
Peptides
CEF
MD01-004_MANA 01
MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
MD01-004_MANA 07
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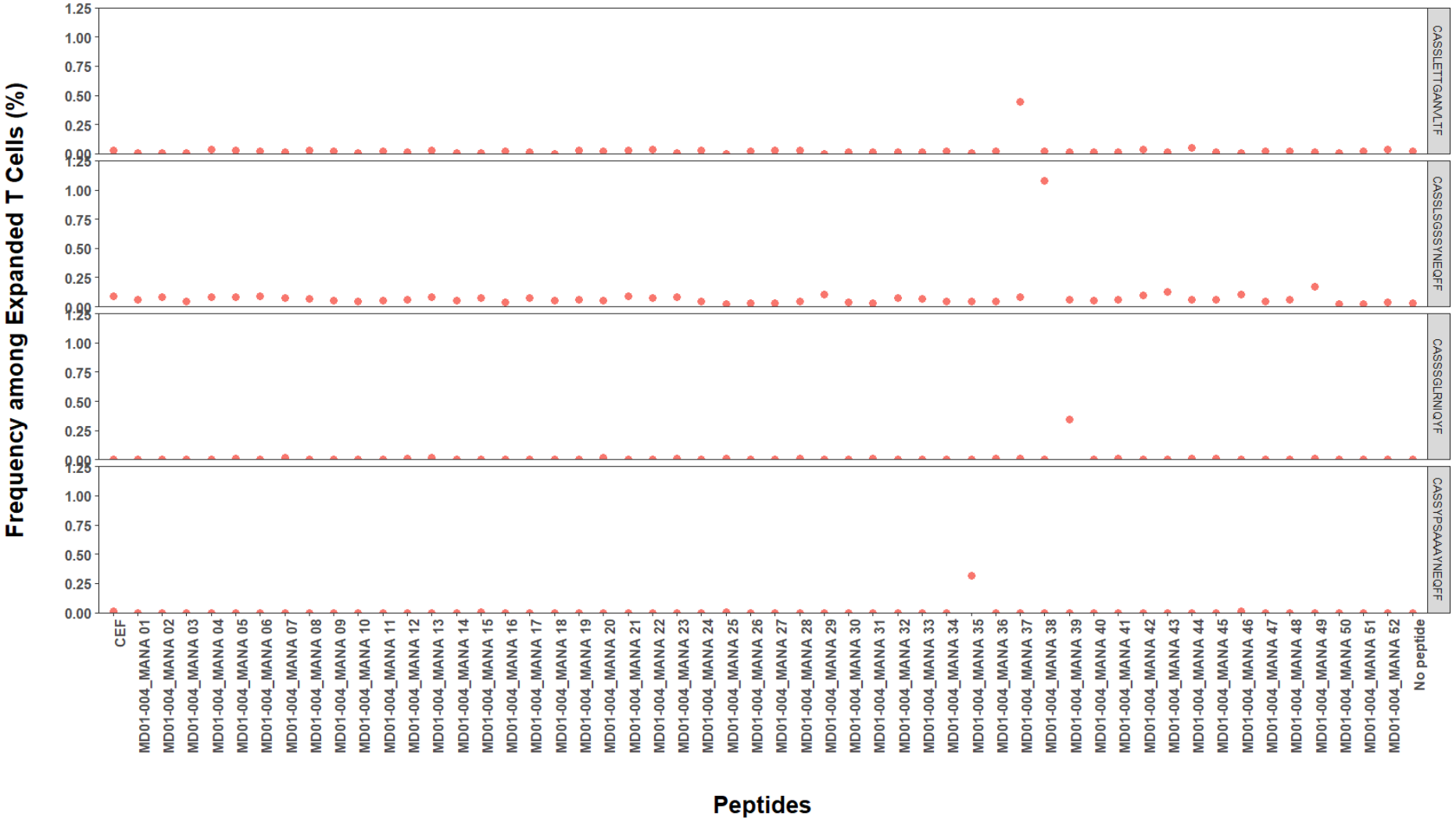
Frequency among Expanded T Cells (%)
1.25
1.00
0.75
0.50
0.25
0.00
CASSLETTGANVLTF
CASSLSGSSYNEQFF
CASSSGLRNIQYF
CASSYPSAAAYNEQFF
CEF
MD01-004_MANA 01
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MD01-004_MANA 25
MD01-004_MANA 26
MD01-004_MANA 27
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MD01-004_MANA 29
MD01-004_MANA 30
MD01-004_MANA 31
MD01-004_MANA 32
MD01-004_MANA 33
MD01-004_MANA 34
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MD01-004_MANA 47
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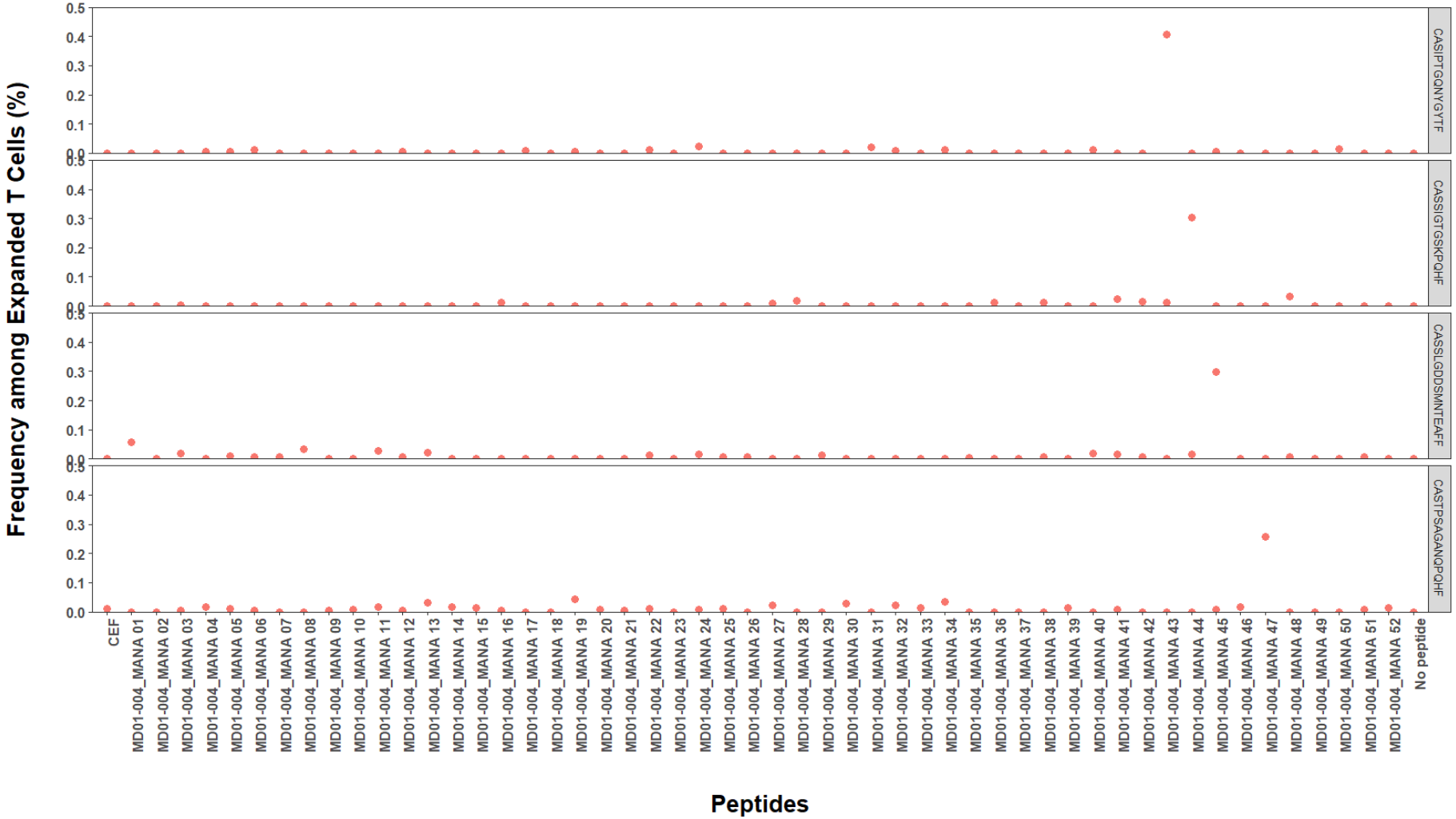
MD01-004_MANA 49
MD01-004_MANA 50
MD01-004_MANA 51
MD01-004_MANA 52
No peptide
Peptides


Frequency among Expanded T Cells (%)
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0.3
0.2
0.1
0.0
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CASSIGTGSKPQHF
CASSLGDDSMNTEAFF
CASTPSAGANQPQHF
CEF
MD01-004_MANA 01
MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
MD01-004_MANA 07
MD01-004_MANA 08
MD01-004_MANA 09
MD01-004_MANA 10
MD01-004_MANA 11
MD01-004_MANA 12
MD01-004_MANA 13
MD01-004_MANA 14
MD01-004_MANA 15
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MD01-004_MANA 47
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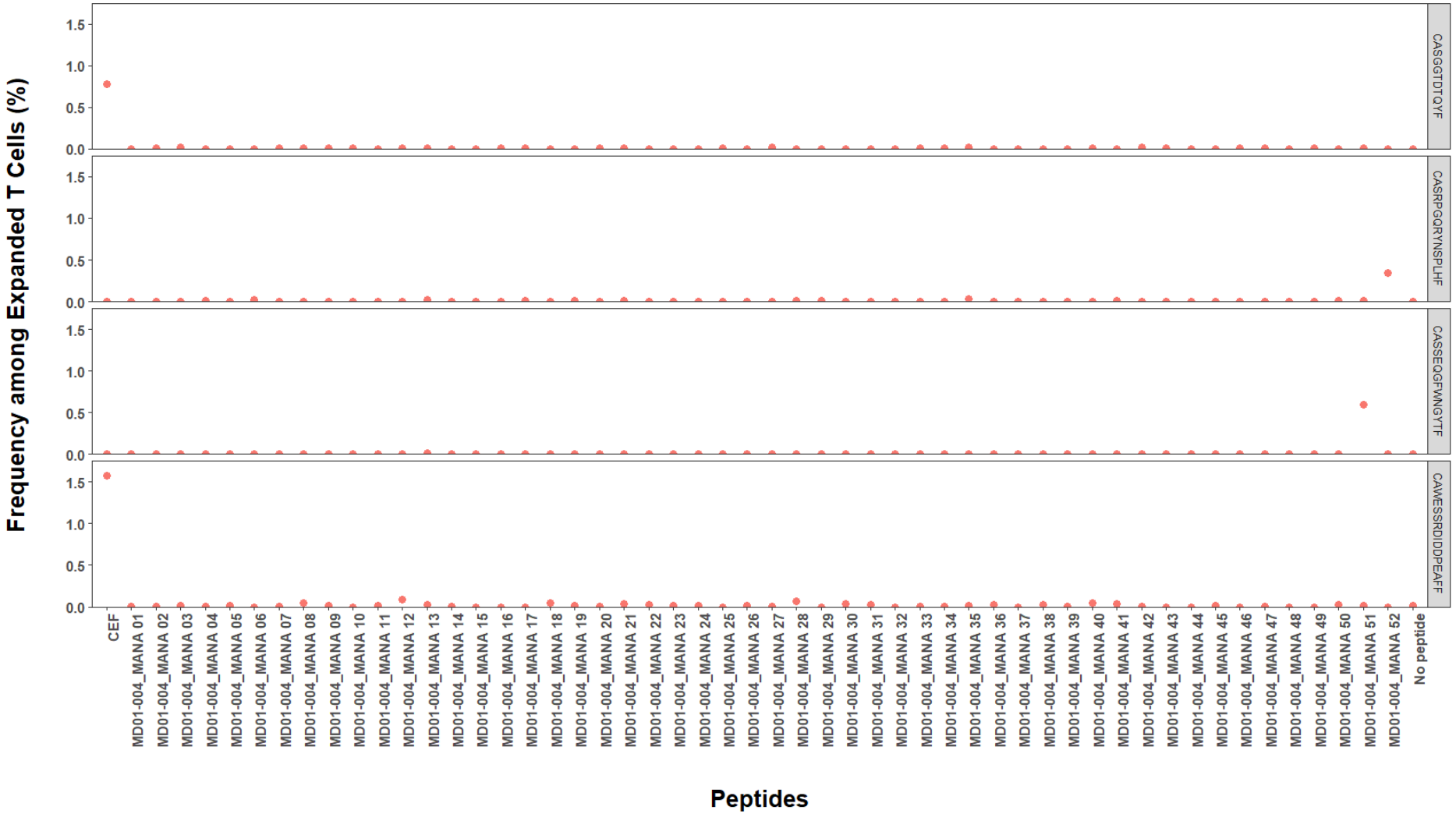
MD01-004_MANA 49
MD01-004_MANA 50
MD01-004_MANA 51
MD01-004_MANA 52
No peptide
Peptides


Frequency among Expanded T Cells (%)
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0.5
1.0
1.5
CASGGTDTQYF
CASRPGQRYNSPLHF
CASSEQGFWNGYTF
CAWESSRDIDDPEAFF
CEF
MD01-004_MANA 01
MD01-004_MANA 02
MD01-004_MANA 03
MD01-004_MANA 04
MD01-004_MANA 05
MD01-004_MANA 06
MD01-004_MANA 07
MD01-004_MANA 08
MD01-004_MANA 09
MD01-004_MANA 10
MD01-004_MANA 11
MD01-004_MANA 12
MD01-004_MANA 13
MD01-004_MANA 14
MD01-004_MANA 15
MD01-004_MANA 16
MD01-004_MANA 17
MD01-004_MANA 18
MD01-004_MANA 19
MD01-004_MANA 20
MD01-004_MANA 21
MD01-004_MANA 22
MD01-004_MANA 23
MD01-004_MANA 24
MD01-004_MANA 25
MD01-004_MANA 26
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MD01-004_MANA 30
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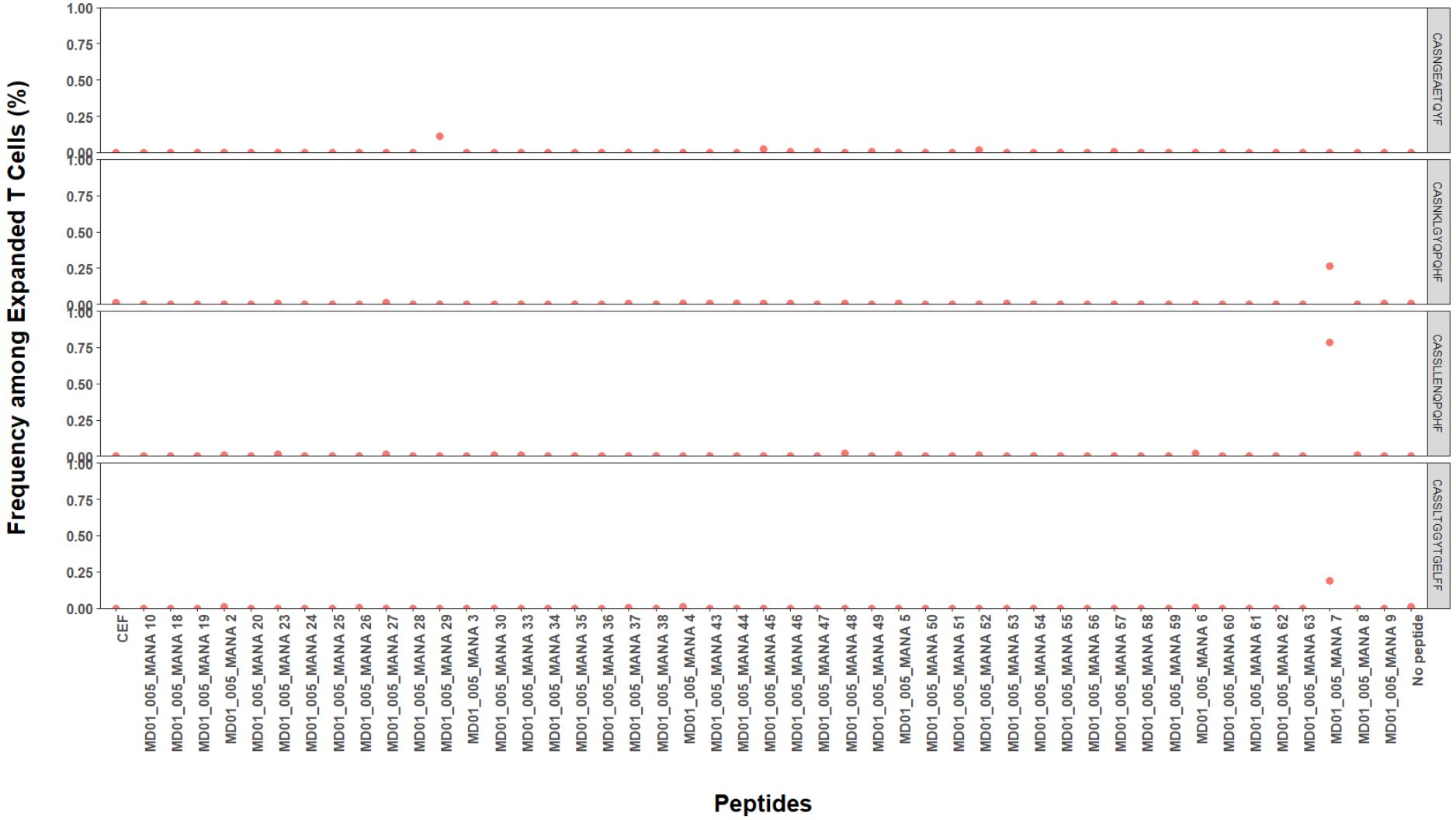
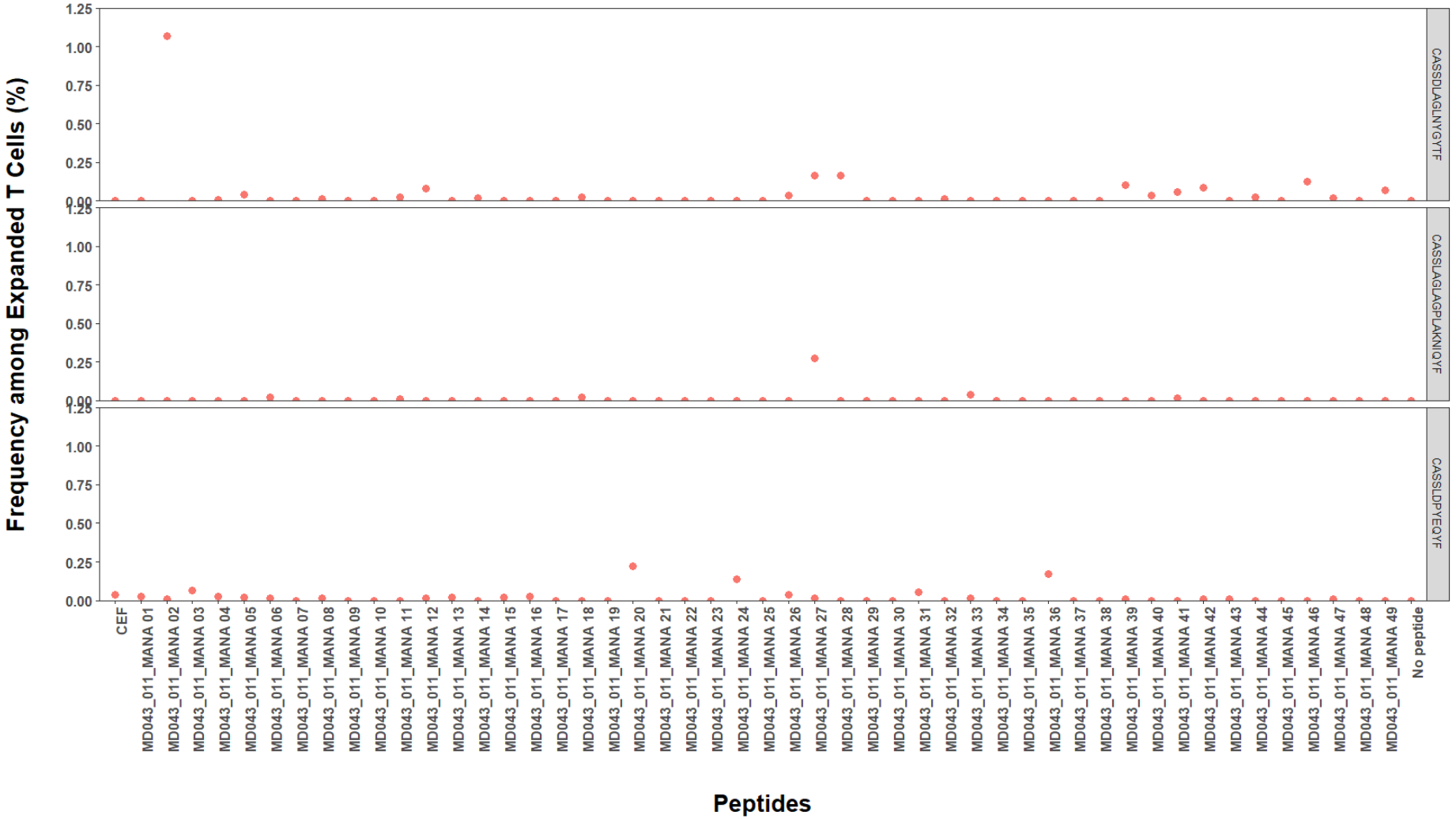
MD01-004_MANA 47
MD01-004_MANA 48
MD01-004_MANA 49
MD01-004_MANA 50
MD01-004_MANA 51
MD01-004_MANA 52
No peptide
Peptides

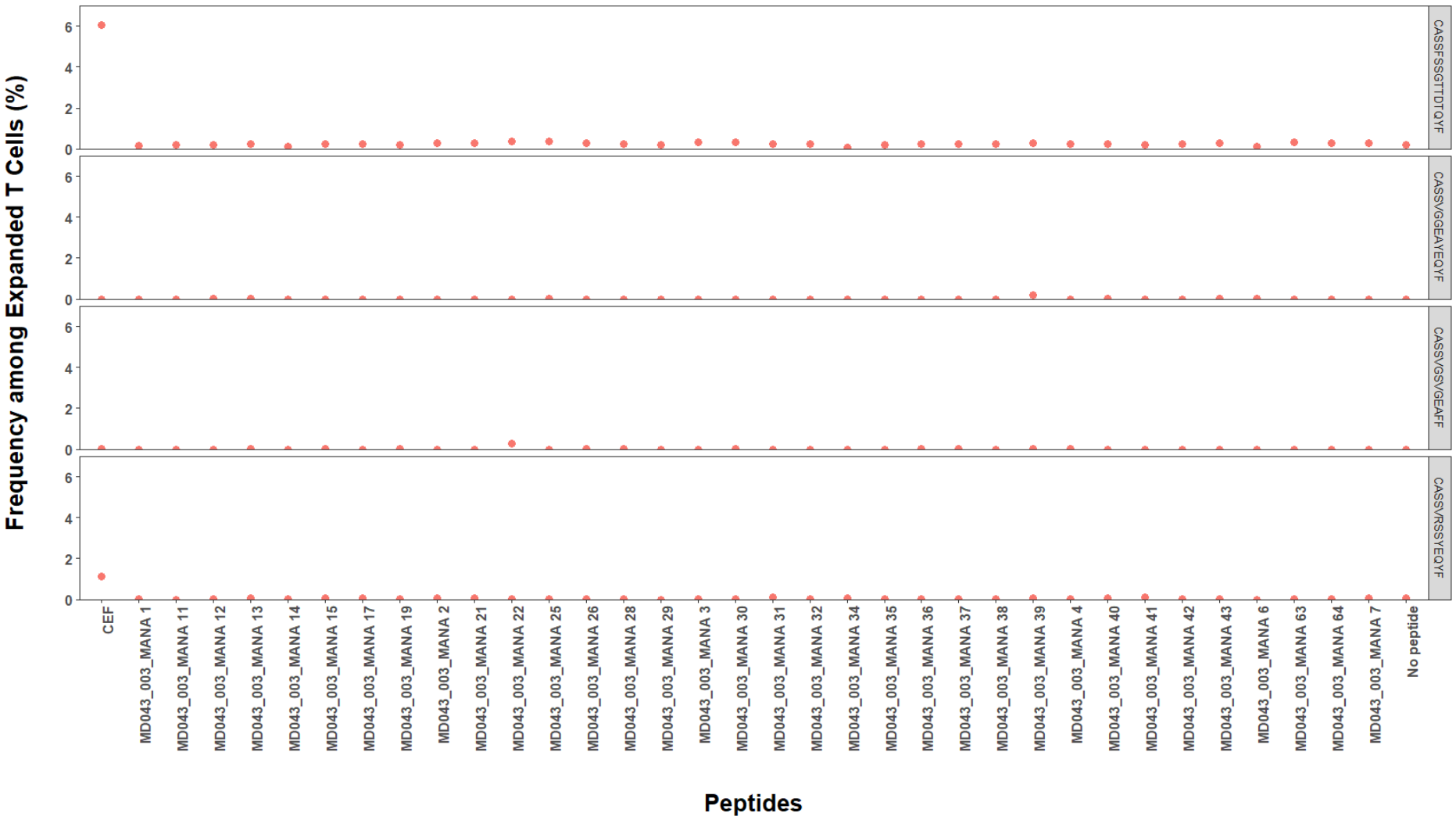
Patient MD01-005

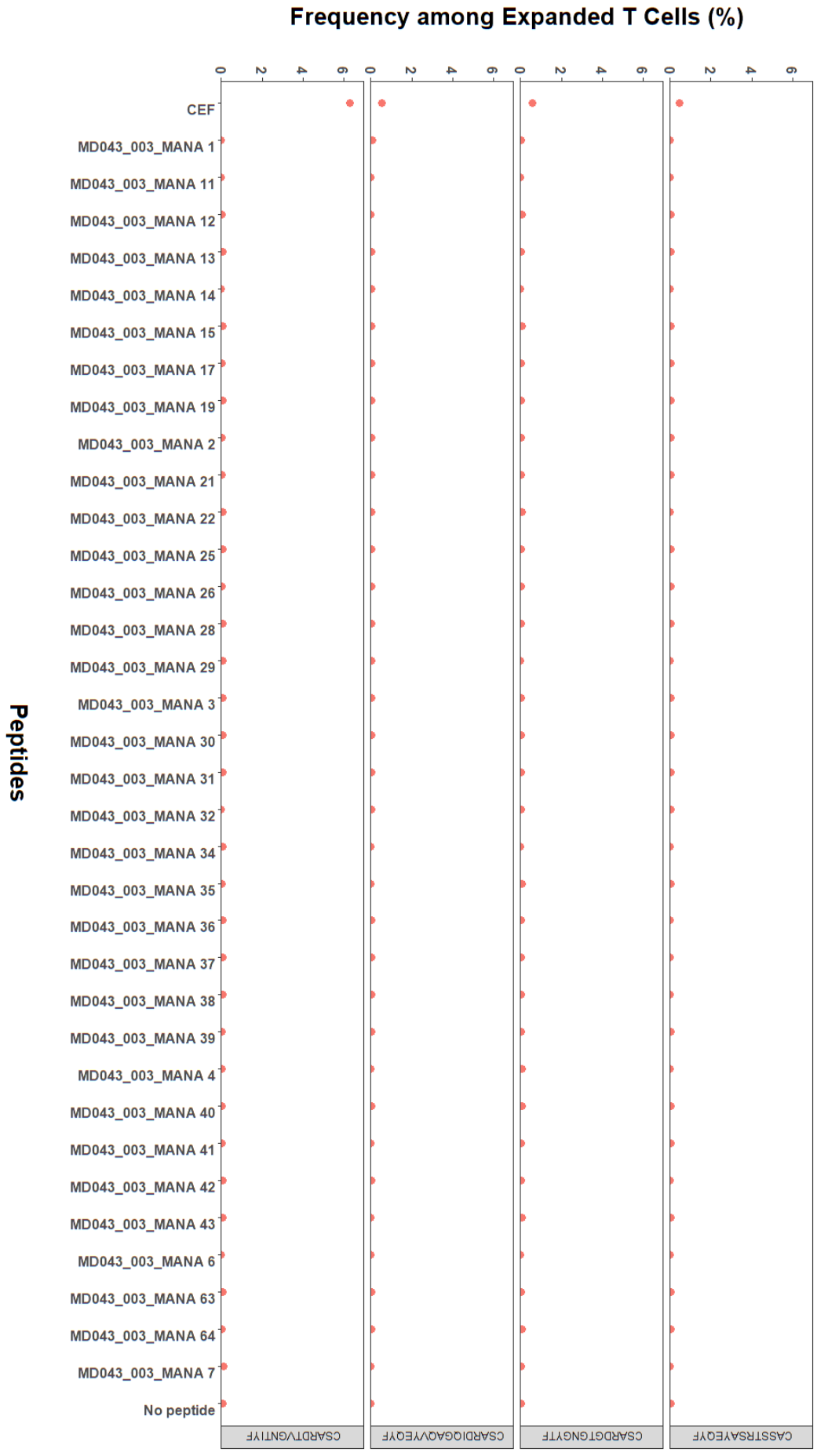


Patient MD043-011



Patient: MD043-003




Frequency among Expanded T Cells (%)
0
2
4
6
Peptides
CEF
MD043_003_MANA 1
MD043_003_MANA 11
MD043_003_MANA 12
MD043_003_MANA 13
MD043_003_MANA 14
MD043_003_MANA 15
MD043_003_MANA 17
MD043_003_MANA 19
MD043_003_MANA 2
MD043_003_MANA 21
MD043_003_MANA 22
MD043_003_MANA 25
MD043_003_MANA 26
MD043_003_MANA 28
MD043_003_MANA 29
MD043_003_MANA 3
MD043_003_MANA 30
MD043_003_MANA 31
MD043_003_MANA 32
MD043_003_MANA 34
MD043_003_MANA 35
MD043_003_MANA 36
MD043_003_MANA 37
MD043_003_MANA 38
MD043_003_MANA 39
MD043_003_MANA 4
MD043_003_MANA 40
MD043_003_MANA 41
MD043_003_MANA 42
MD043_003_MANA 43
MD043_003_MANA 6
MD043_003_MANA 63
MD043_003_MANA 64
MD043_003_MANA 7
No peptide
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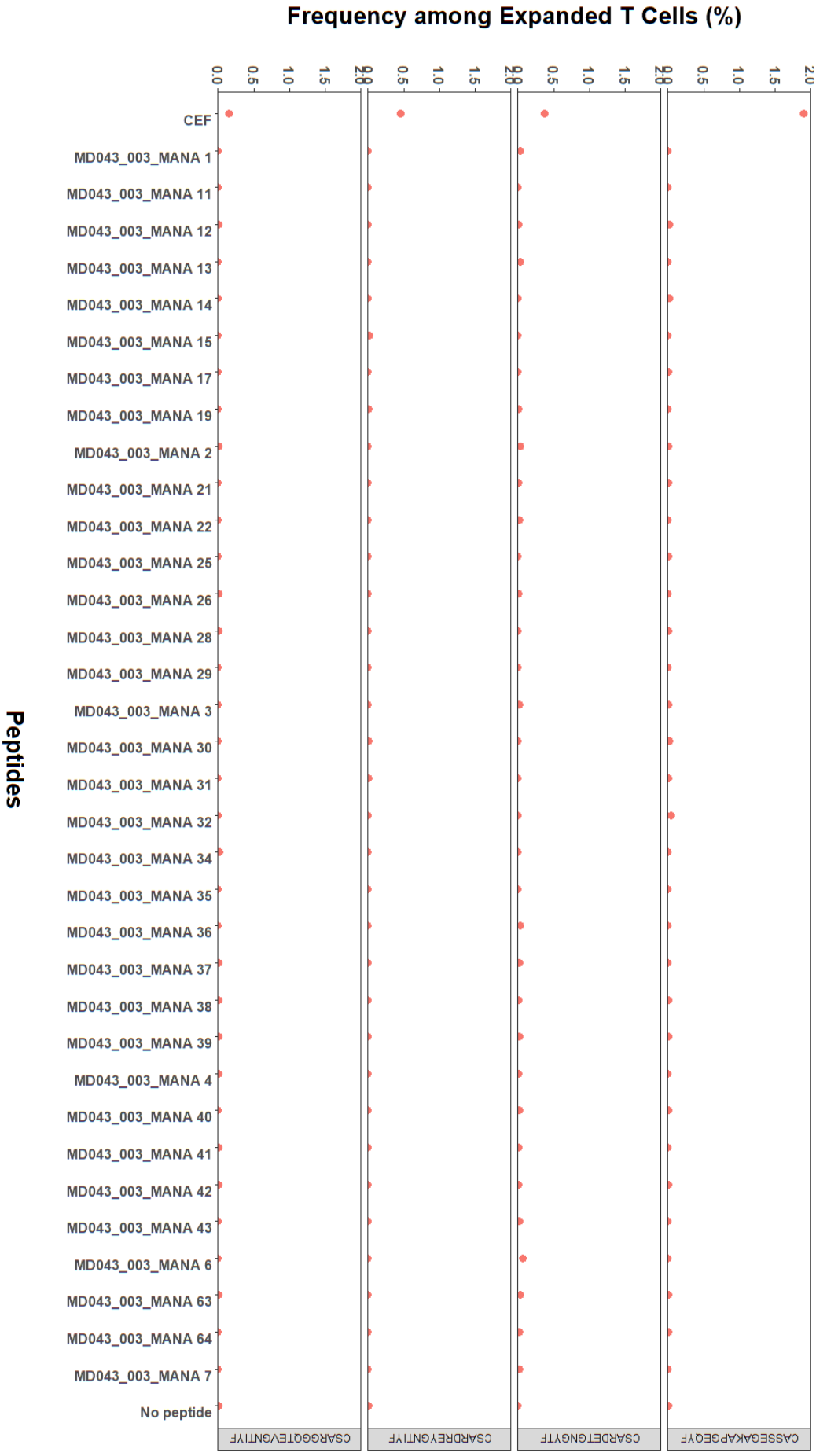
CSARDIGGAQVYEQYF
CSARDGTGNGYTF
CASSTRSAYEQYF


Frequency among Expanded T Cells (%)
0.0
0.5
1.0
1.5
2.0
Peptides
CEF
MD043_003_MANA 1
MD043_003_MANA 11
MD043_003_MANA 12
MD043_003_MANA 13
MD043_003_MANA 14
MD043_003_MANA 15
MD043_003_MANA 17
MD043_003_MANA 19
MD043_003_MANA 2
MD043_003_MANA 21
MD043_003_MANA 22
MD043_003_MANA 25
MD043_003_MANA 26
MD043_003_MANA 28
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MD043_003_MANA 3
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MD043_003_MANA 31
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MD043_003_MANA 36
MD043_003_MANA 37
MD043_003_MANA 38
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MD043_003_MANA 4
MD043_003_MANA 40
MD043_003_MANA 41
MD043_003_MANA 42
MD043_003_MANA 43
MD043_003_MANA 6
MD043_003_MANA 63
MD043_003_MANA 64
MD043_003_MANA 7
No peptide
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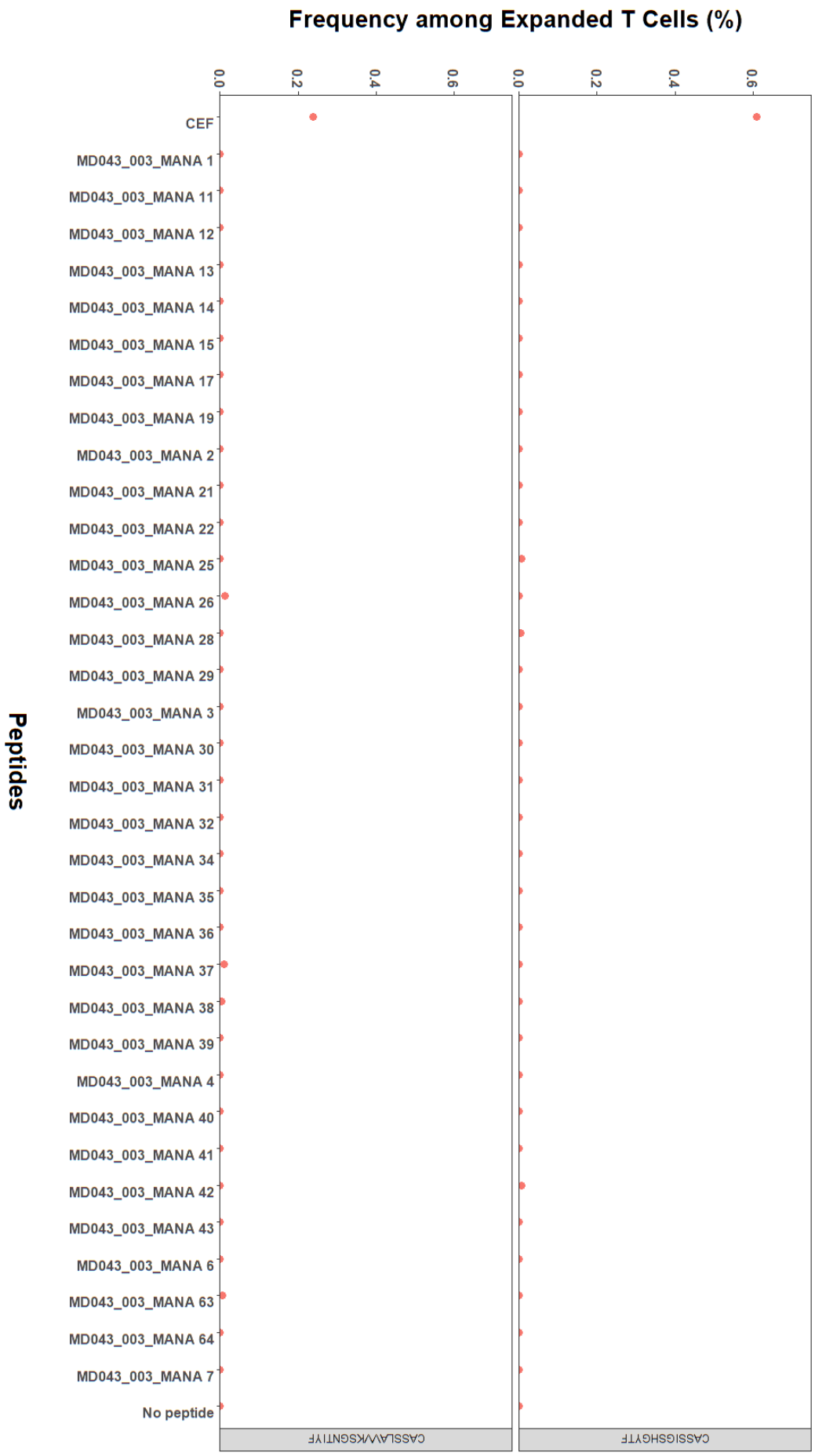
CSARDREYGNTIYF
CSARDETGNGYTF
CASSEGAKAPGEQYF


Frequency among Expanded T Cells (%)
0.0
0.2
0.4
0.6
Peptides
CEF
MD043_003_MANA 1
MD043_003_MANA 11
MD043_003_MANA 12
MD043_003_MANA 13
MD043_003_MANA 14
MD043_003_MANA 15
MD043_003_MANA 17
MD043_003_MANA 19
MD043_003_MANA 2
MD043_003_MANA 21
MD043_003_MANA 22
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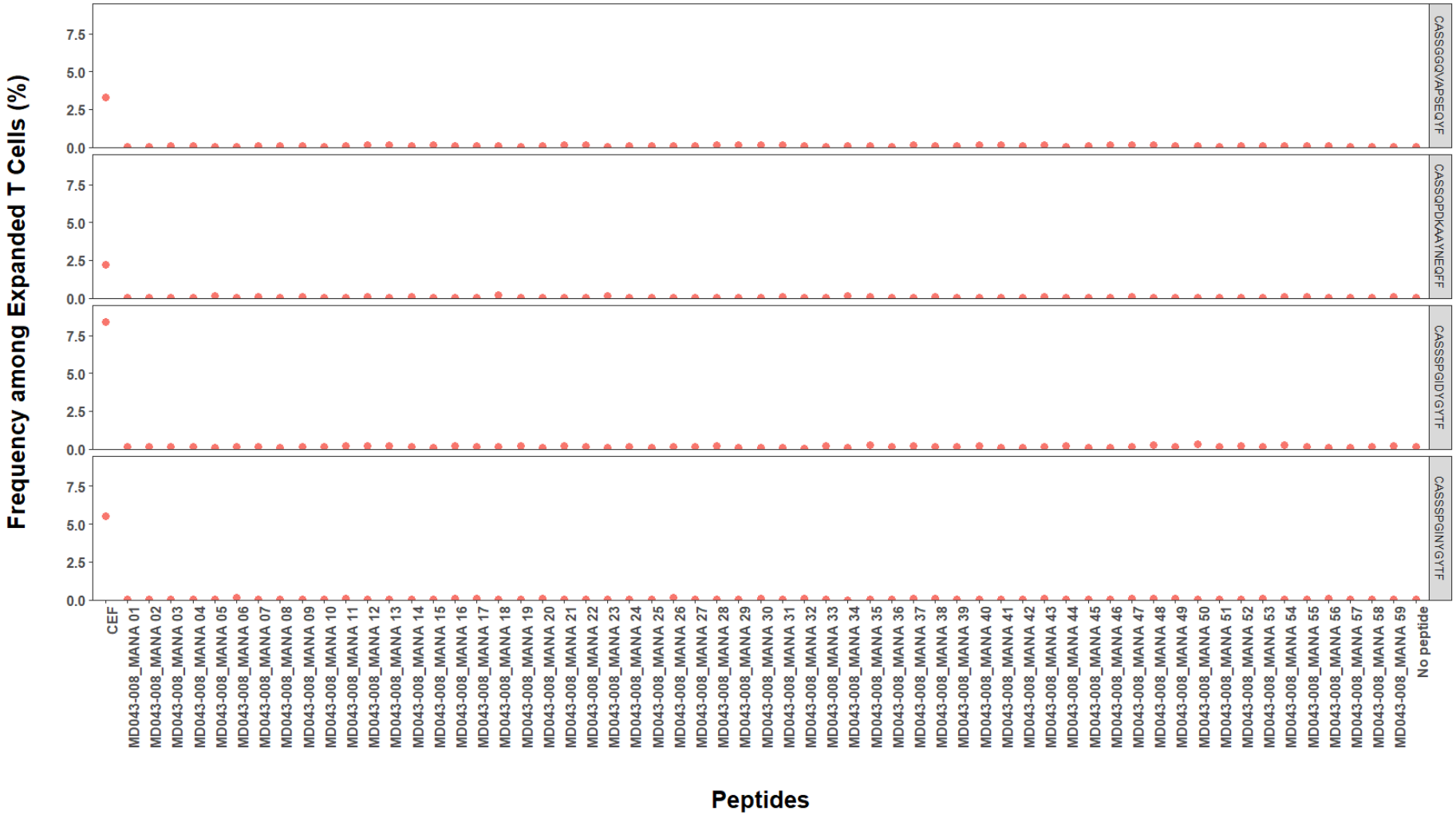
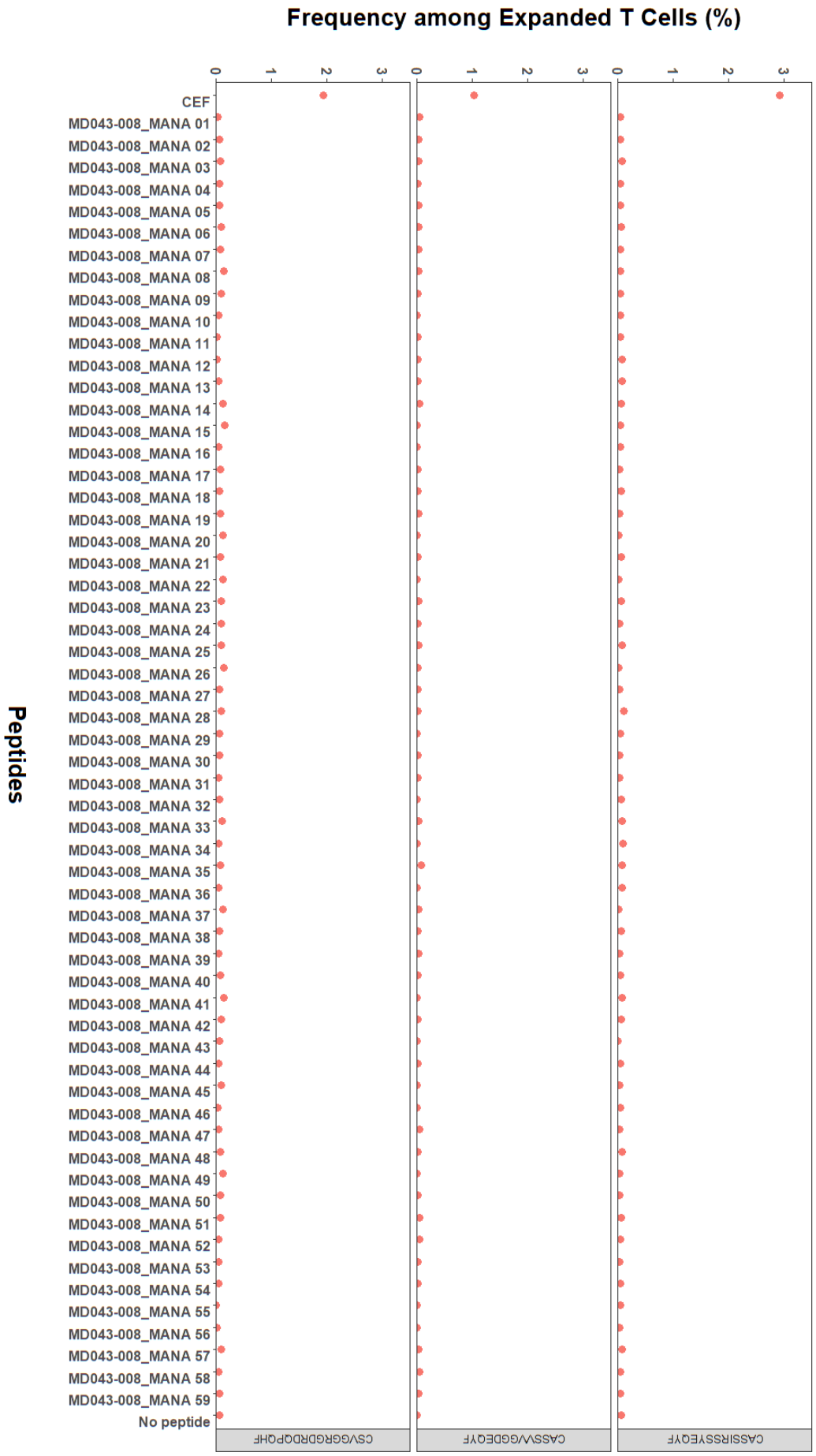
MD043_003_MANA 40
MD043_003_MANA 41
MD043_003_MANA 42
MD043_003_MANA 43
MD043_003_MANA 6
MD043_003_MANA 63
MD043_003_MANA 64
MD043_003_MANA 7
No peptide
CASSLAVVKSGNTIYF
CASSIGSHGYTF

Patient MD043-008




Frequency among Expanded T Cells (%)
Peptides
CEF
MD043-008_MANA 01
MD043-008_MANA 02
MD043-008_MANA 03
MD043-008_MANA 04
MD043-008_MANA 05
MD043-008_MANA 06
MD043-008_MANA 07
MD043-008_MANA 08
MD043-008_MANA 09
MD043-008_MANA 10
MD043-008_MANA 11
MD043-008_MANA 12
MD043-008_MANA 13
MD043-008_MANA 14
MD043-008_MANA 15
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MD043-008_MANA 17
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MD043-008_MANA 19
MD043-008_MANA 20
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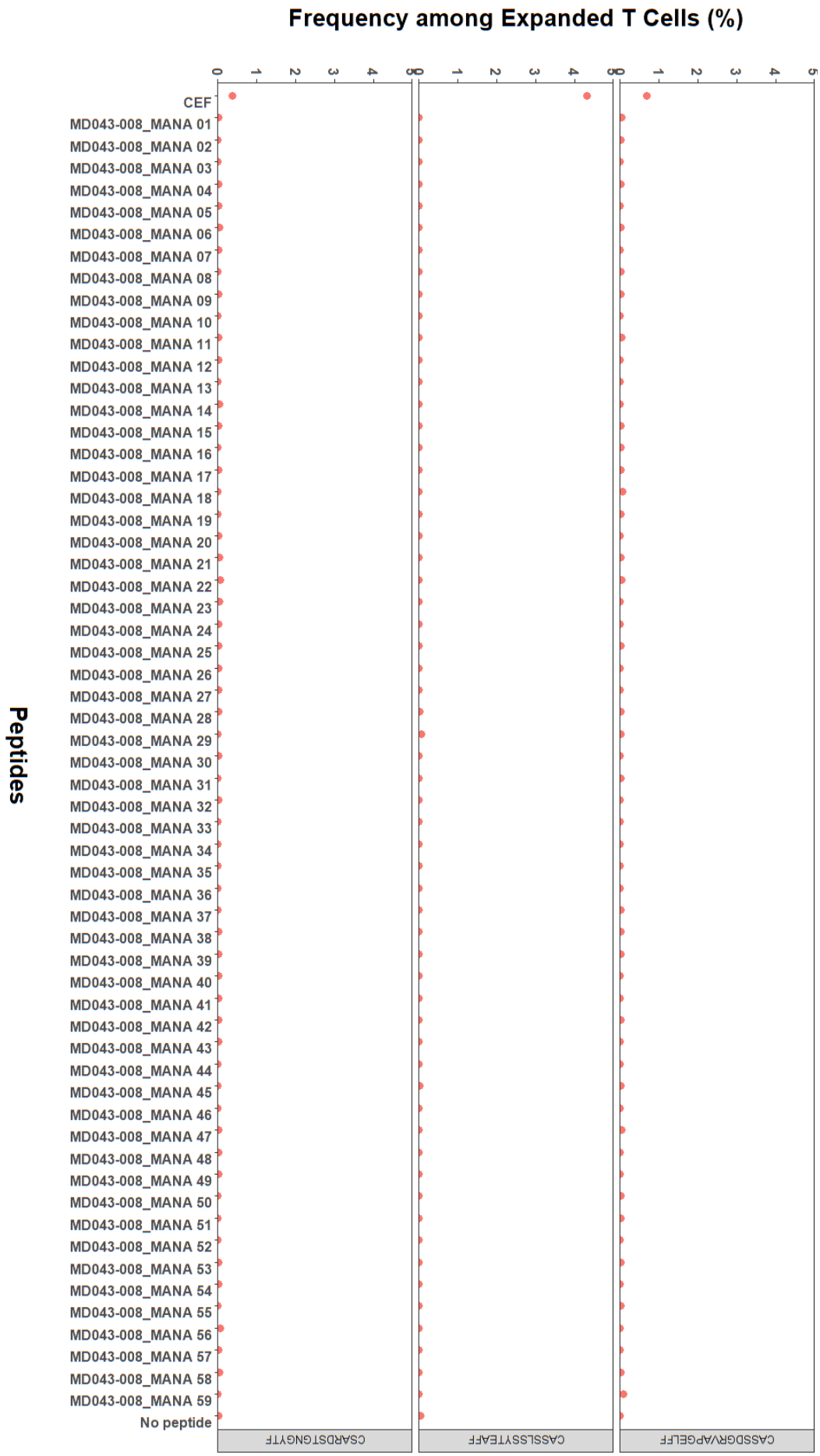
MD043-008_MANA 54
MD043-008_MANA 55
MD043-008_MANA 56
MD043-008_MANA 57
MD043-008_MANA 58
MD043-008_MANA 59
No peptide
0
1
2
3
CSVGGRGDRDGPGHF
CASSVVGGDEQYF
CASSIRSSYEQYF


Frequency among Expanded T Cells (%)
Peptides
CEF
MD043-008_MANA 01
MD043-008_MANA 02
MD043-008_MANA 03
MD043-008_MANA 04
MD043-008_MANA 05
MD043-008_MANA 06
MD043-008_MANA 07
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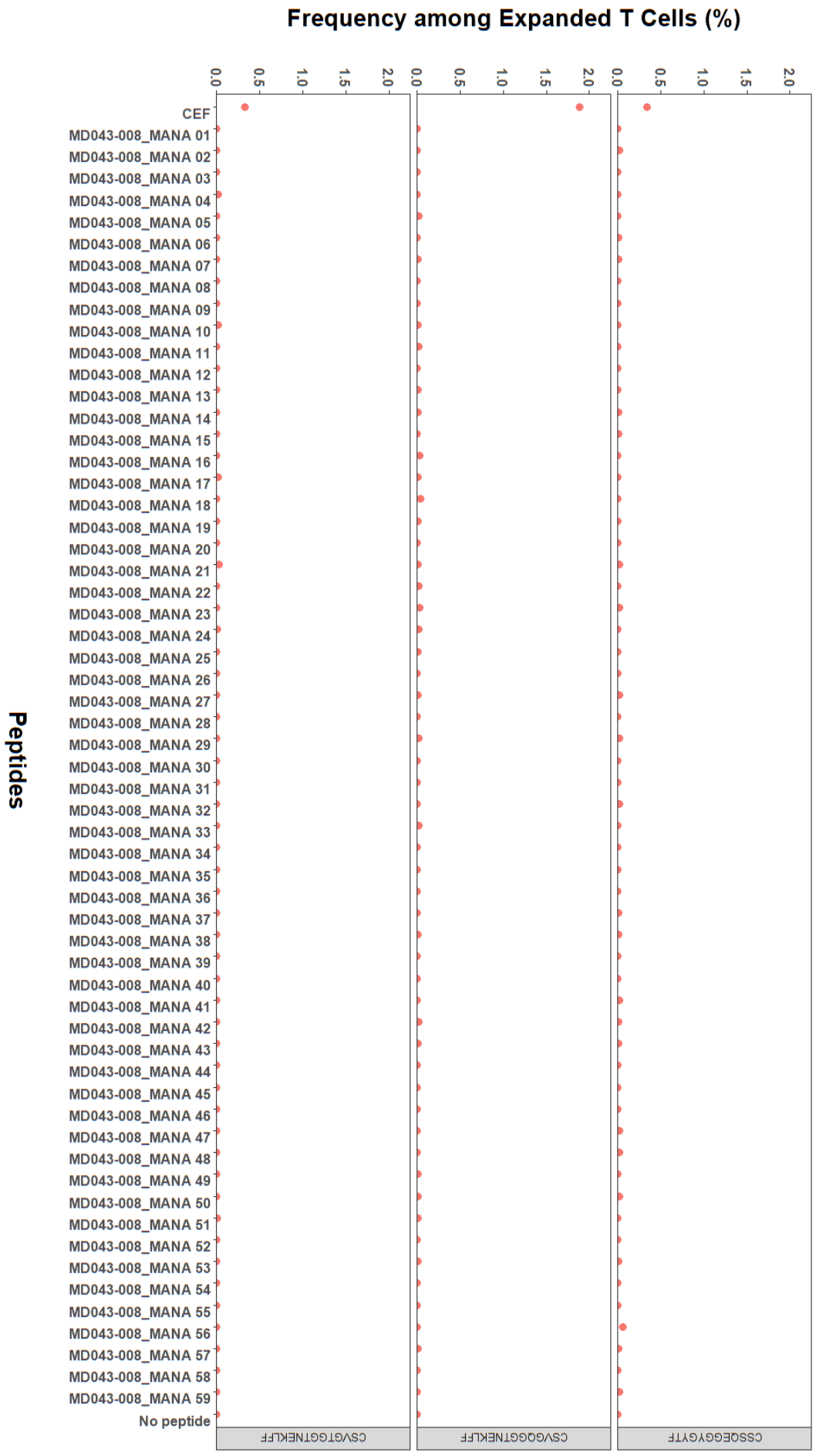
MD043-008_MANA 55
MD043-008_MANA 56
MD043-008_MANA 57
MD043-008_MANA 58
MD043-008_MANA 59
No peptide
0
1
2
3
4
5
CSARDSTGNGYTF
CASSLSSYTEAFF
CASSDGRVAPGELFF


Frequency among Expanded T Cells (%)
0.0
0.5
1.0
1.5
2.0
Peptides
CEF
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MD043-008_MANA 02
MD043-008_MANA 03
MD043-008_MANA 04
MD043-008_MANA 05
MD043-008_MANA 06
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MD043-008_MANA 56
MD043-008_MANA 57
MD043-008_MANA 58
MD043-008_MANA 59
No peptide
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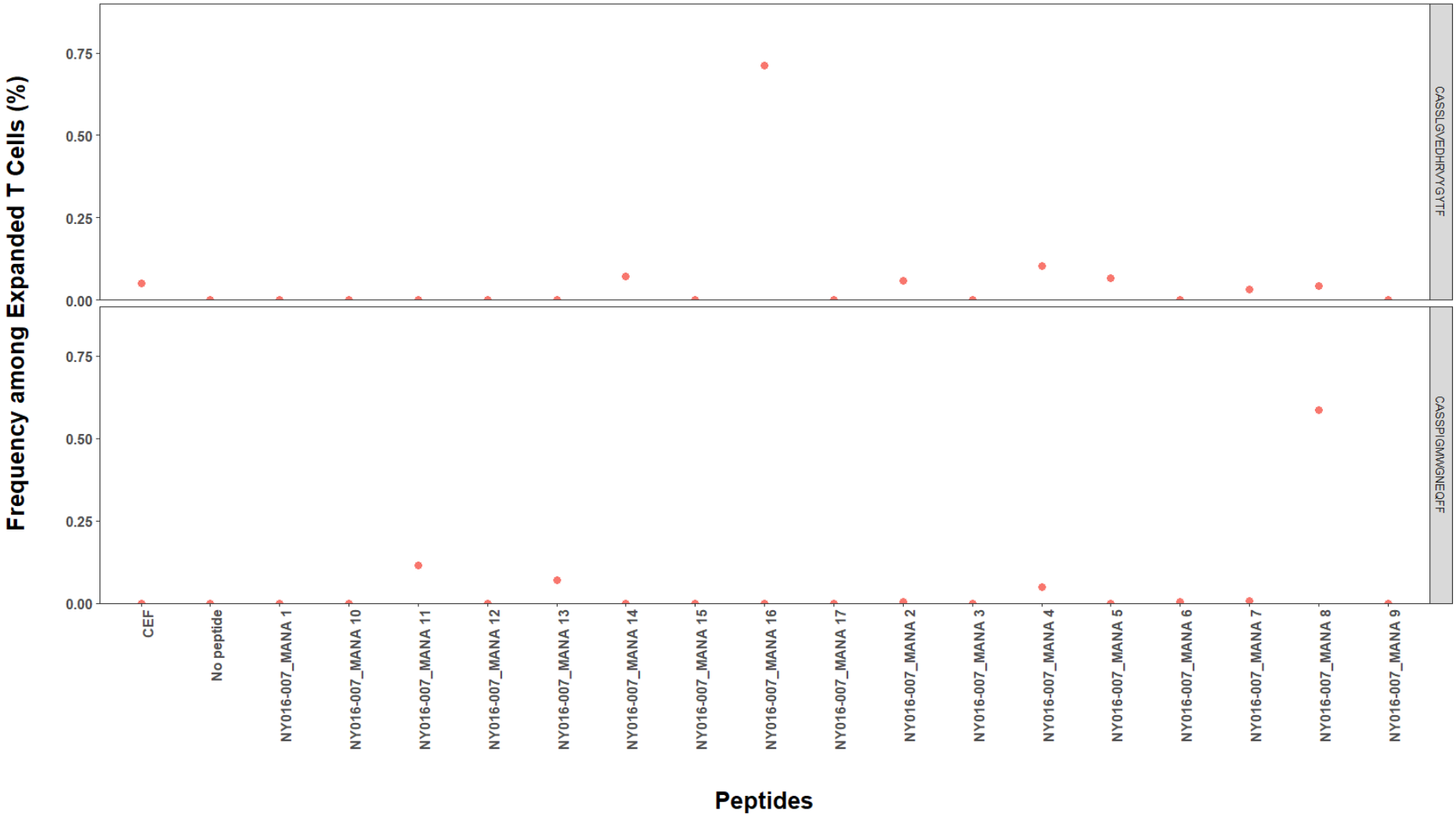
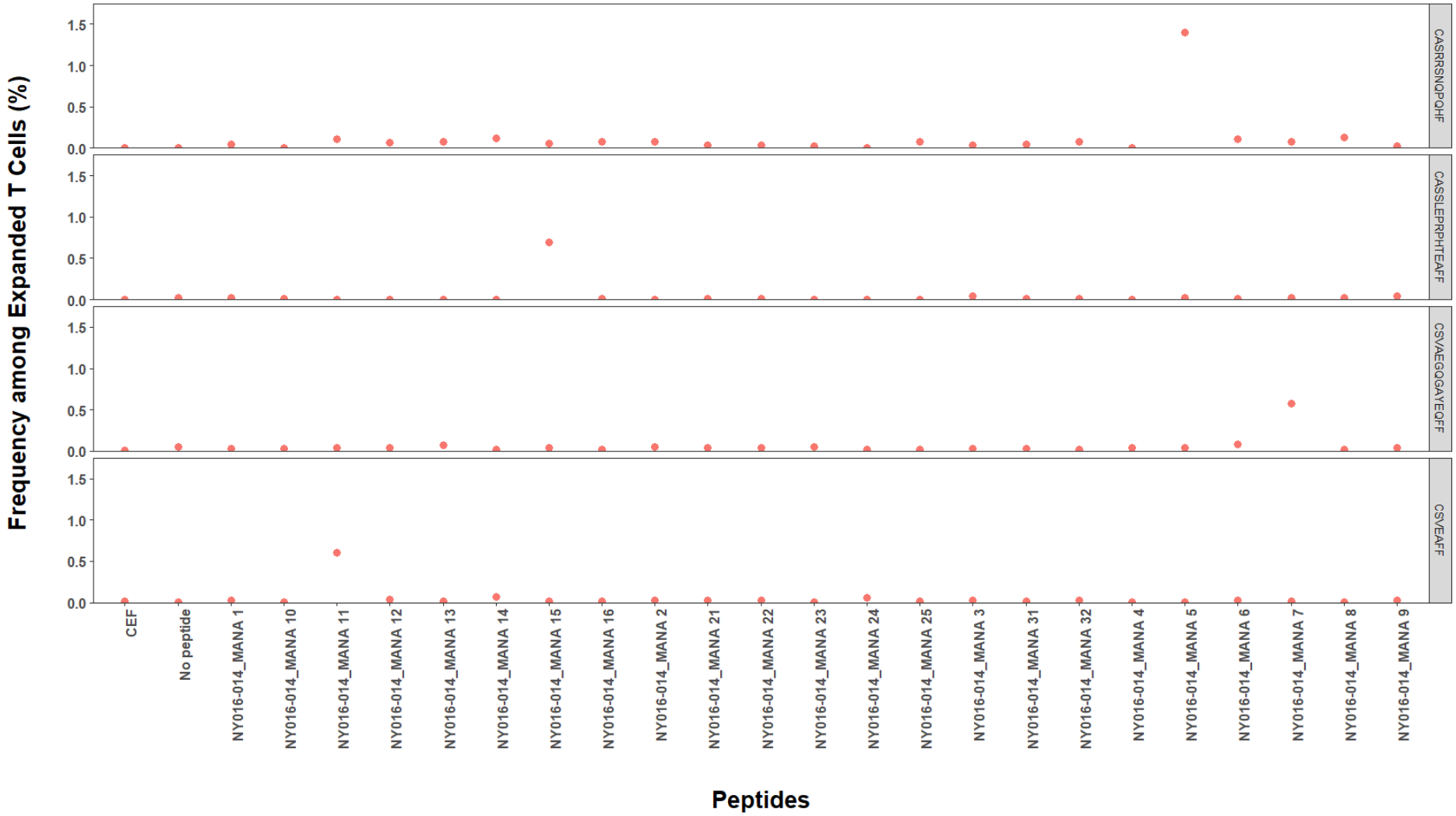
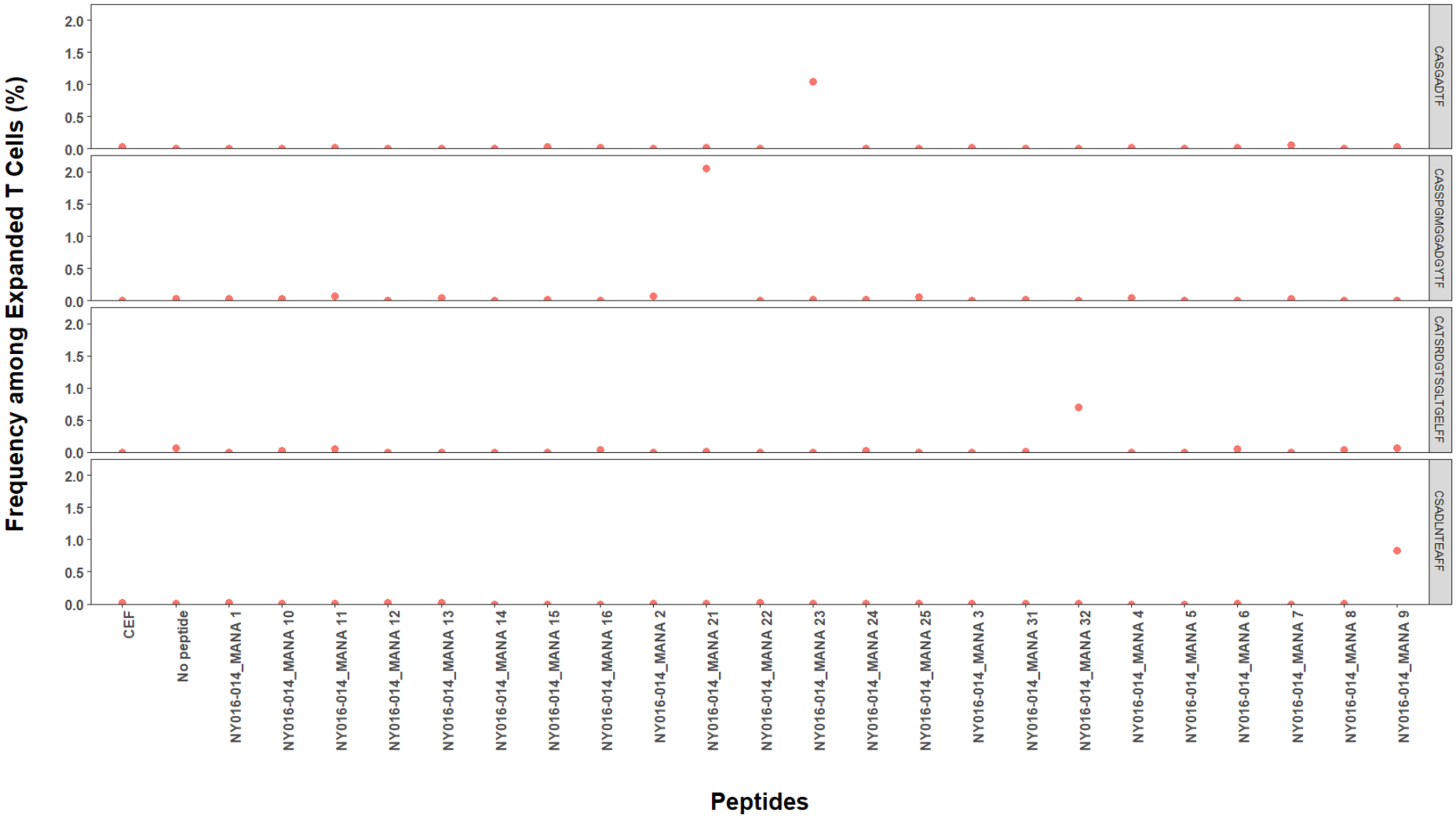
CSVGQGGTNEKLFF
CSSQEGGYGYTF

Patient NY016-007



Patient NY016-014




Frequency among Expanded T Cells (%)
CASGADTF
CASSPGMGGADGYTF
CATSRDGTSGLTGELFF
CSADLNTEAFF
0.0
0.5
1.0
1.5
2.0
CEF
No peptide
NY016-014_MANA 1
NY016-014_MANA 10
NY016-014_MANA 11
NY016-014_MANA 12
NY016-014_MANA 13
NY016-014_MANA 14
NY016-014_MANA 15
NY016-014_MANA 16
NY016-014_MANA 2
NY016-014_MANA 21
NY016-014_MANA 22
NY016-014_MANA 23
NY016-014_MANA 24
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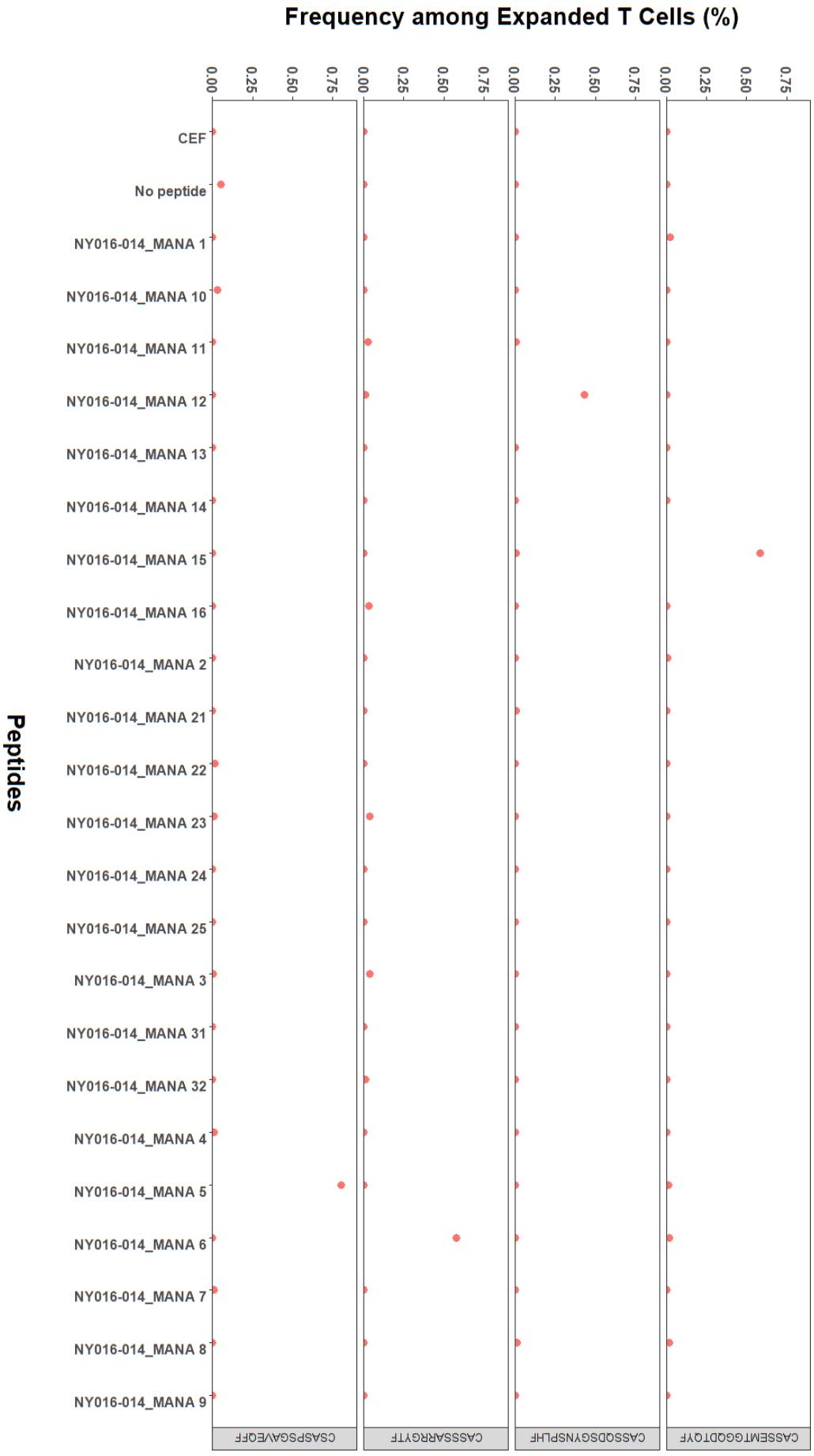
NY016-014_MANA 3
NY016-014_MANA 31
NY016-014_MANA 32
NY016-014_MANA 4
NY016-014_MANA 5
NY016-014_MANA 6
NY016-014_MANA 7
NY016-014_MANA 8
NY016-014_MANA 9
Peptides


Frequency among Expanded T Cells (%)
0.00
0.25
0.50
0.75
Peptides
CEF
No peptide
NY016-014_MANA 1
NY016-014_MANA 10
NY016-014_MANA 11
NY016-014_MANA 12
NY016-014_MANA 13
NY016-014_MANA 14
NY016-014_MANA 15
NY016-014_MANA 16
NY016-014_MANA 2
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NY016-014_MANA 31
NY016-014_MANA 32
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NY016-014_MANA 6
NY016-014_MANA 7
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NY016-014_MANA 9
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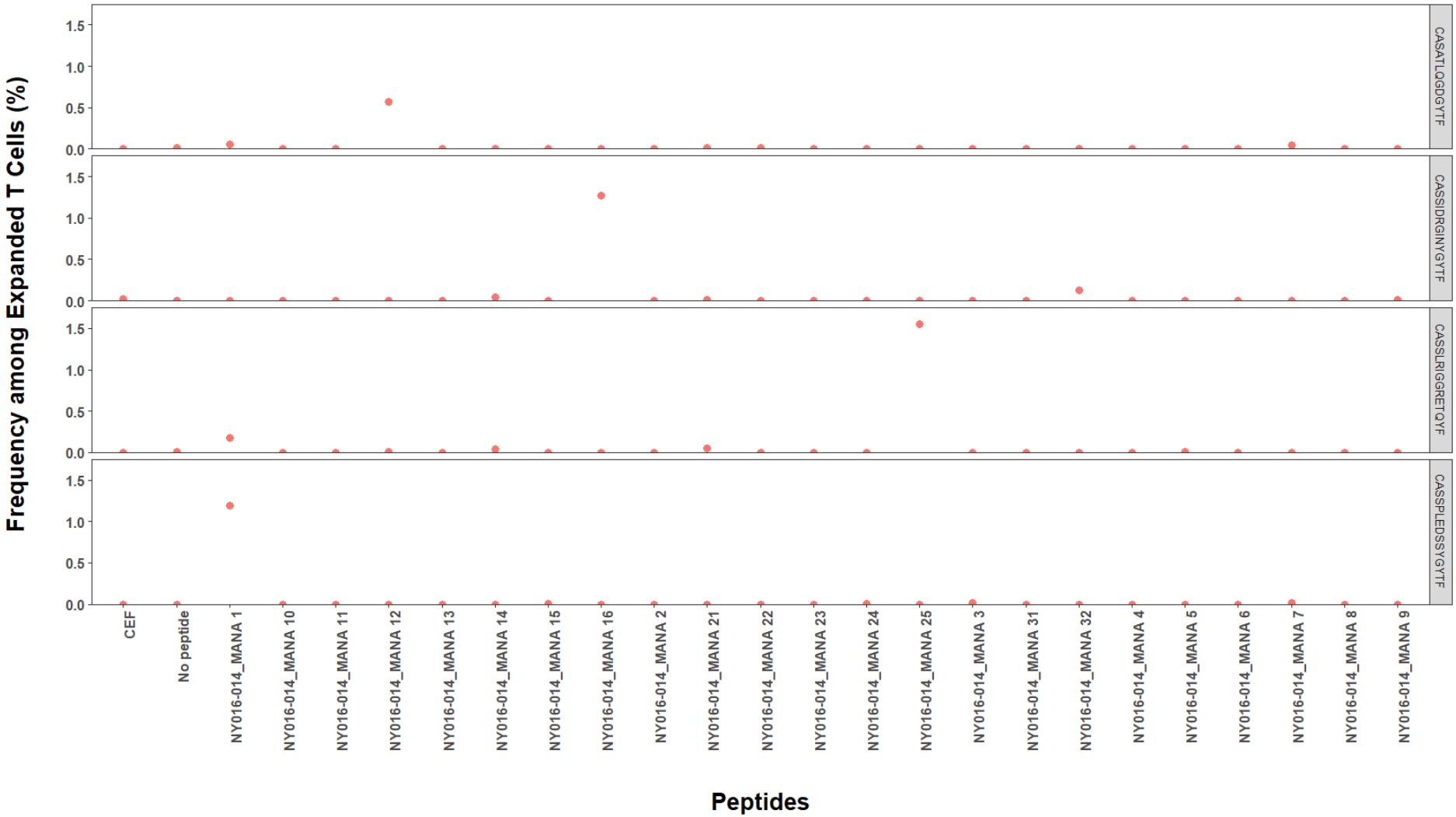
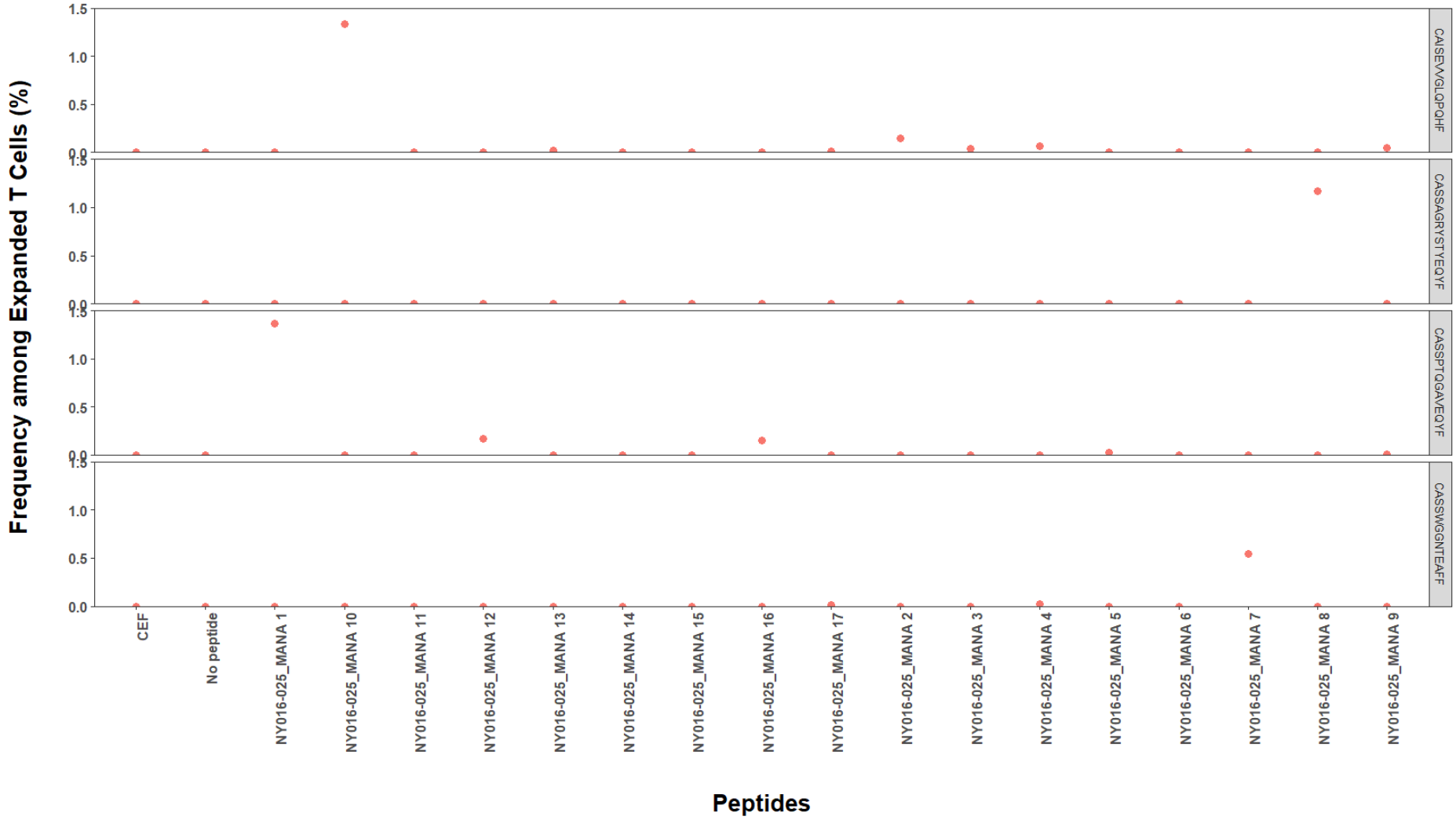
CASSQDSGYNSPLHF
CASSEMTGGDTQYF


Frequency among Expanded T Cells (%)
1.5
1.0
0.5
0.0
CASATLQGDGYTF
CASSIDRGINYGYTF
CASSLRIGGRETQYF
CASSPLEDSSYGYTF
CEF
No peptide
NY016-014_MANA 1
NY016-014_MANA 10
NY016-014_MANA 11
NY016-014_MANA 12
NY016-014_MANA 13
NY016-014_MANA 14
NY016-014_MANA 15
NY016-014_MANA 16
NY016-014_MANA 2
NY016-014_MANA 21
NY016-014_MANA 22
NY016-014_MANA 23
NY016-014_MANA 24
NY016-014_MANA 25
NY016-014_MANA 3
NY016-014_MANA 31
NY016-014_MANA 32
NY016-014_MANA 4
NY016-014_MANA 5
NY016-014_MANA 6
NY016-014_MANA 7
NY016-014_MANA 8
NY016-014_MANA 9
Peptides

Patient NY016-025