Supplemental Data for

Peptide length determines the outcome of T cell receptor/peptide-MHCI engagement

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[*]These authors contributed equally to this work.

SD Figures S1 to S7

SD Table S1 & S2

SD References

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: MHCI-peptide length preference correlates with length of the "index" peptide.
6×10^4 target cells expressing either HLA A*0201 or HLA B*3501 were pulsed in duplicate with the following "sizing scan" mixtures (1mM) for 2 hours at 37^{o}C: x^8, x^9, x^{10}, x^{11}, x^{12} and x^{13} (where x is any of the 19 proteogenic L-amino acids excluding cysteine). Subsequently, 3×10^4 ILA1 (**A**), 1E6 (**B**), SB14 (**C**), SB10 (**D**) or SB27 (**E**) $CD8^+$ T-cells were added and incubated overnight. The supernatant was then harvested and assayed for MIP1β by ELISA. Error bars represent SDs, data are representative of a minimum of three separate experiments.

Figure S2: The unique MHCI-peptide length footprint of ILA1.
6×10^4 target cells expressing HLA A*0201 were pulsed in duplicate with mixtures from 8mer (**A**), 9mer (**B**), 10mer (**C**), 11mer (**D**), 12mer (**E**) or 13mer (**F**) CPL scans (100 μM) at 37^{o}C. After 2 hours, 3×10^4 ILA1 $CD8^+$ T-cells were added and incubated overnight. The supernatant was then harvested and assayed for MIP1β by ELISA. Error bars represent SDs, data are representative of a minimum of three separate experiments.

Figure S3: The unique MHCI-peptide length footprint of 1E6.
6×10^4 target cells expressing HLA A*0201 were pulsed in duplicate with mixtures from 8mer (**A**), 9mer (**B**), 10mer (**C**), 11mer (**D**), 12mer (**E**) or 13mer (**F**) CPL scans (100 μM) at 37^{o}C. After 2 hours, 3×10^4 1E6 $CD8^+$ T-cells were added and incubated overnight. The supernatant was then harvested and assayed for MIP1β by ELISA. Error bars represent SDs, data are representative of a minimum of three separate experiments.

Figure S4: The unique MHCI-peptide length footprint of MEL5.
6×10^4 target cells expressing HLA A*0201 were pulsed in duplicate with mixtures from 8mer (**A**), 9mer (**B**), 10mer (**C**), 11mer (**D**), 12mer (**E**) or 13mer (**F**) CPL scans (100 μM) at 37^{o}C. After 2 hours, 3×10^4 MEL5 $CD8^+$ T-cells were added and incubated overnight. The supernatant was then harvested and assayed for MIP1β by ELISA. Error bars represent SDs, data are representative of a minimum of three separate experiments.

Figure S5: The unique MHCI-peptide length footprint of SB27.
6×10^4 target cells expressing HLA B*3508 were pulsed in duplicate with mixtures from 8mer (**A**), 9mer (**B**), 10mer (**C**), 11mer (**D**), 12mer (**E**) or 13mer (**F**) CPL scans (100 μM) at 37^{o}C.

After 2 hours, 3×10^4 SB27 $CD8^+$ T-cells were added and incubated overnight. The supernatant was then harvested and assayed for MIP1β by ELISA. Error bars represent SDs, data are representative of a minimum of three separate experiments.

Figure S6: Sizing scan mixtures of all lengths are capable of binding HLA A*0201.
0.5 x 10^6 T2 cells were incubated in RPMI 1640 with either 100 μM HPVGEADYFEY (non-HLA A*0201 binder), or various concentrations of the indicated sizing scan mixture (x^8, x^9, x^{10}, x^{11}, x^{12} or x^{13}) at 26°C for 14–16 hours, then at 37°C for 2 hours (A) or a further 2 hours at 37°C (B) before staining for HLA A*0201 surface expression. Duplicate samples were acquired for each condition using a FACSCantoII flow cytometer. Data were analyzed with FlowJo software. DMSO controls at the appropriate concentration for each peptide or peptide mixture subsequently stained for HLA A*0201 surface expression were used to subtract background from each sample. Error bars represent SDs. Although, it is interesting to note that a hierarchy of binding begins to appear at lower concentrations and under melting conditions, it is important to note that sizing scan mixtures are not used under these conditions in the main study.

Figure S7: The CDR1α loop of the MEL5 TCR lies closely over the N-terminus of the ELA peptide. The interactions between the CDR1α loop of the MEL5 TCR (shown as yellow sticks) and the N-terminus of the ELAGIGILTV peptide (shown as blue sticks) are shown. Van der Waals interactions (4Å cut-off) are depicted as red dotted lines and hydrogen bonds (3.4Å cut-off) are shown as black dotted lines. Unusually for a TCR/pMHC interaction, the MEL5 TCR makes a number of important contacts with the N-terminus of the peptide. These contacts include hydrogen bonds between the TCR CDR1α residue Gln31 and peptide residues Glu1, Leu2, Gly4 and Ile5.

SUPPLEMENTAL TABLE LEGENDS

Table S1: 1E6 recognition of 11mer and 12mer peptides sampled from a large peptide set selected by CPL-based importance sampling. Simultaneous curve fitting (as described previously[1]) was used to estimate functional sensitivity measured as pEC_{50}.

Table S2: Data collection and refinement statistics (molecular replacement) for HLA A*0201-ILAKFLHRL. PDB code: 414W.

SUPPLEMENTAL REFERENCES

1. Wooldridge L, Ekeruche-Makinde J, van den Berg HA, et al. A single autoimmune T cell receptor recognizes more than a million different peptides. *J Biol Chem*. 2012;287(2):1168-1177.

Figure S1

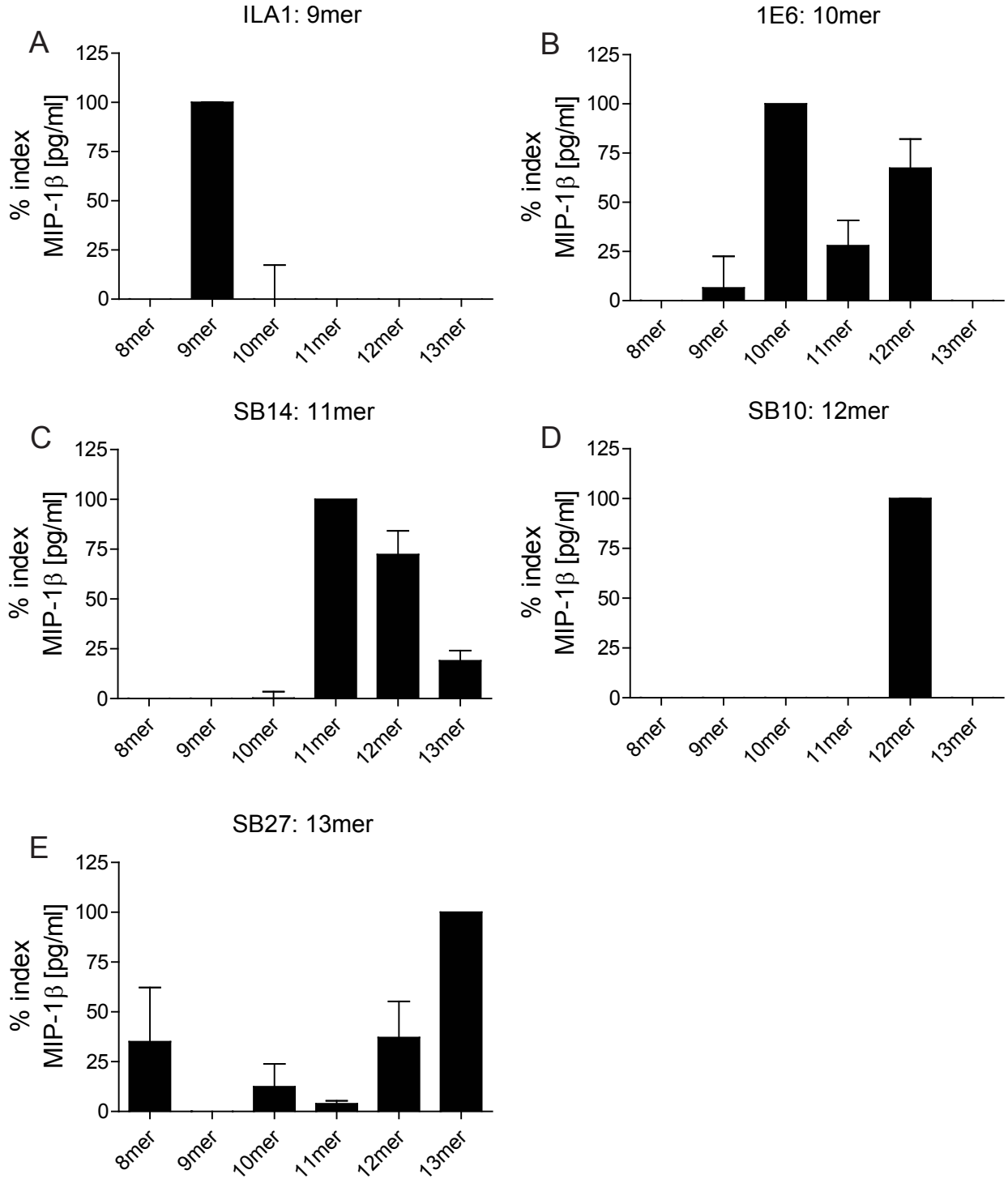

A
ILA1: 9mer
% index MIP-1β [pg/ml]
125
100
75
50
25
0
8mer
9mer
10mer
11mer
12mer
13mer
B
1E6: 10mer
C
SB14: 11mer
D
SB10: 12mer
E
SB27: 13mer

Figure S2A

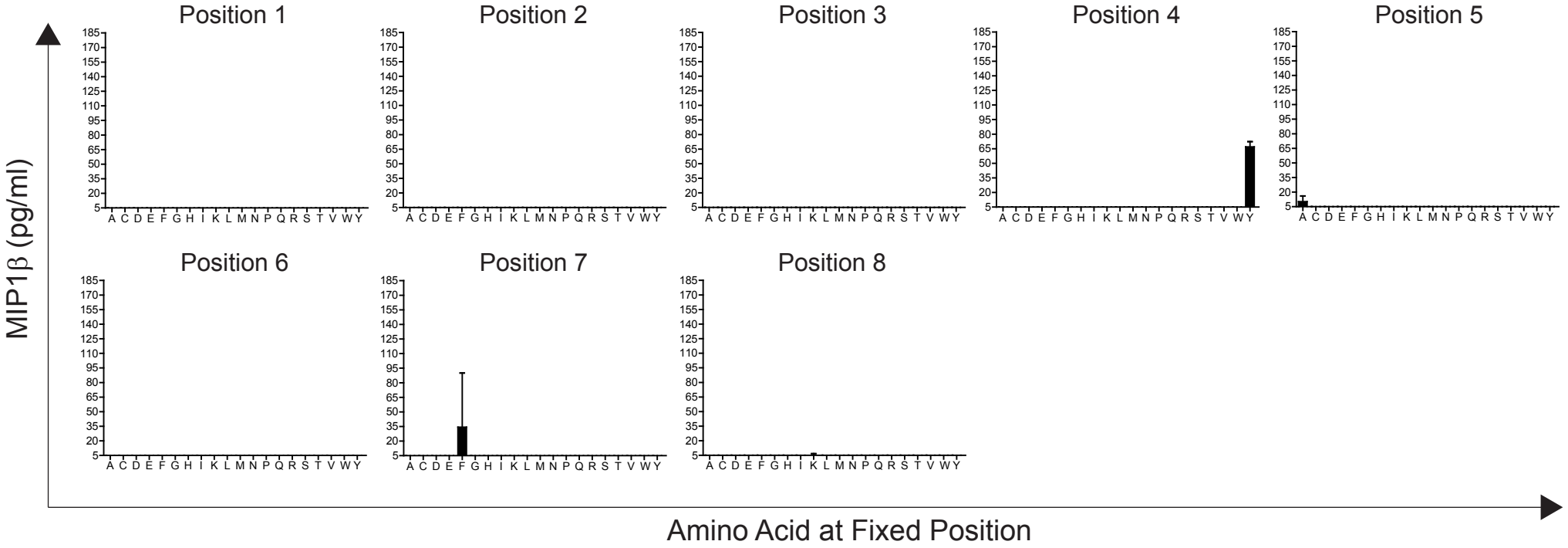
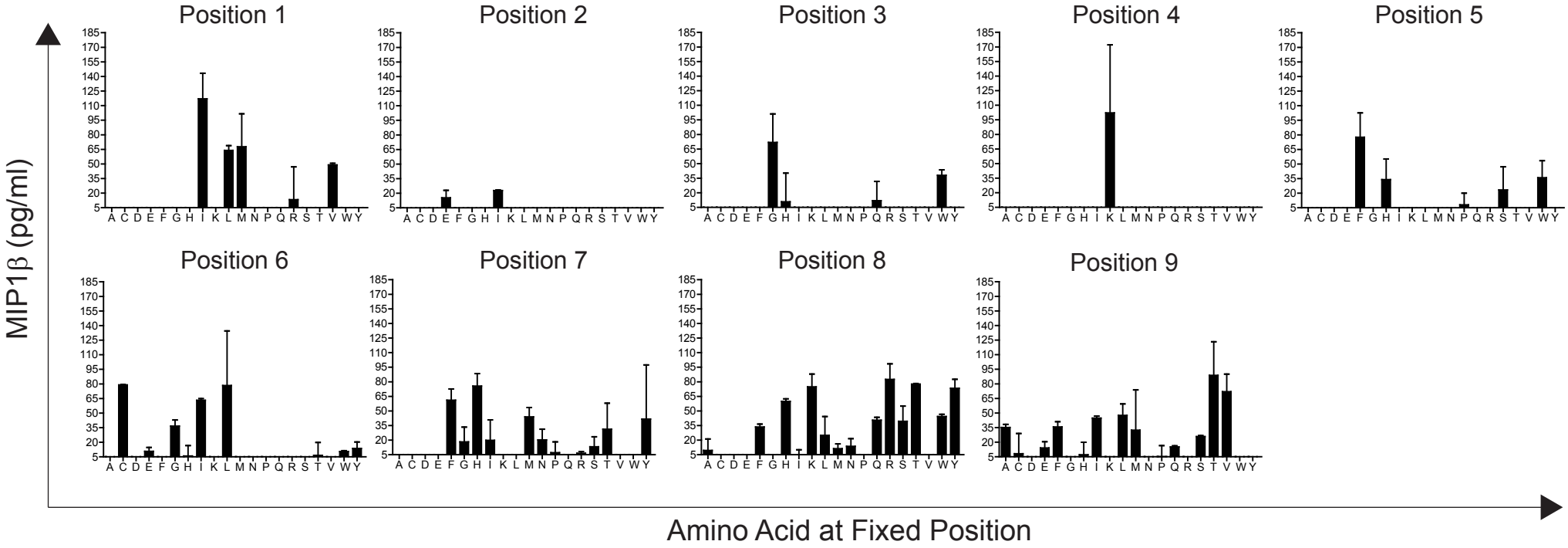

Position 1
Position 2
Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
MIP1β (pg/ml)
185
170
155
140
125
110
95
80
65
50
35
20
5
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid at Fixed Position

Figure S2B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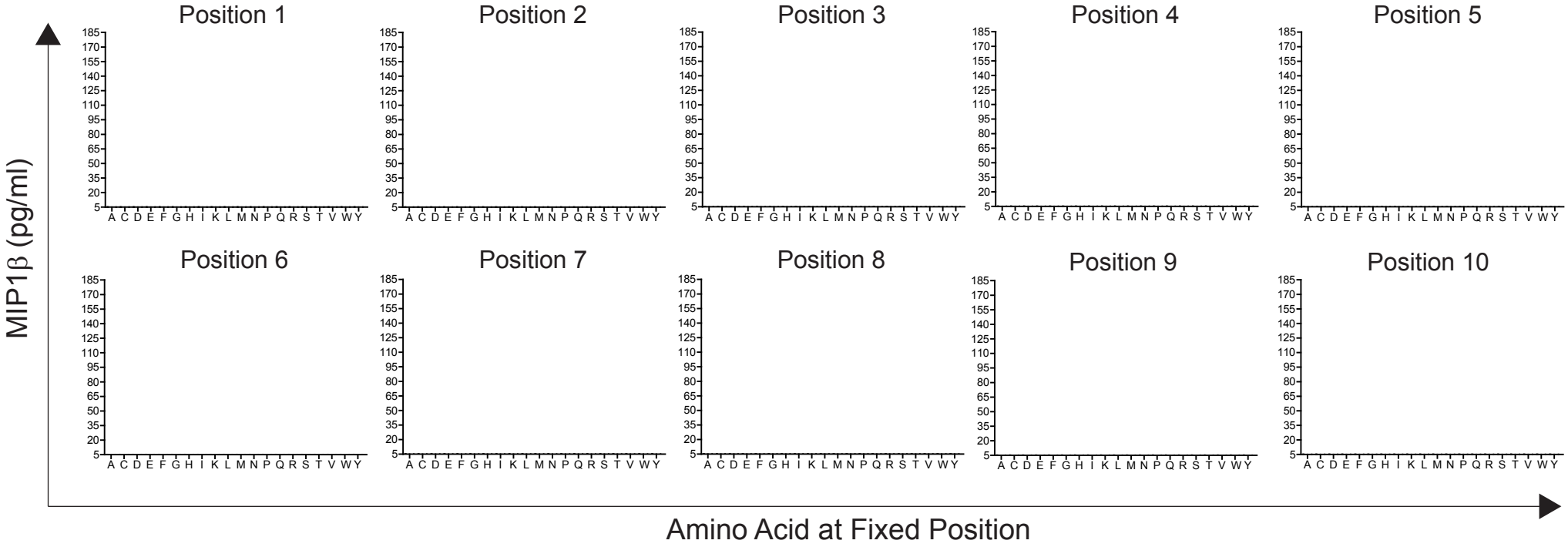
Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
Position 9
MIP1β (pg/ml)
Amino Acid at Fixed Position
A C D E F G H I K L M N P Q R S T V W Y

Figure S2C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Position 7
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Position 9
Position 10
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170
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140
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95
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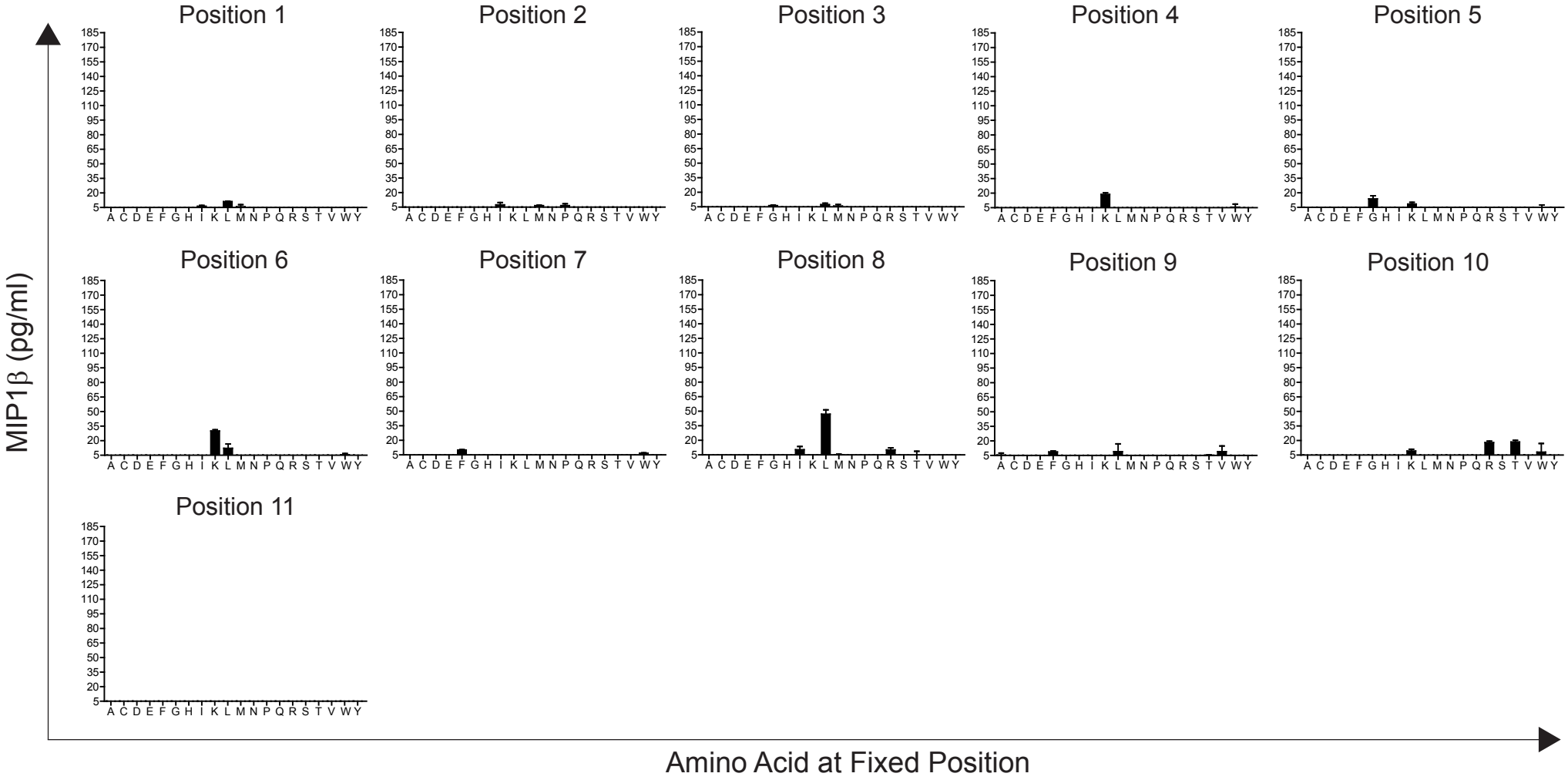
20
5
A C D E F G H I K L M N P Q R S T V W Y
MIP1β (pg/ml)
Amino Acid at Fixed Position

Figure S2D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Position 2
Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
Position 9
Position 10
Position 11
MIP1β (pg/ml)
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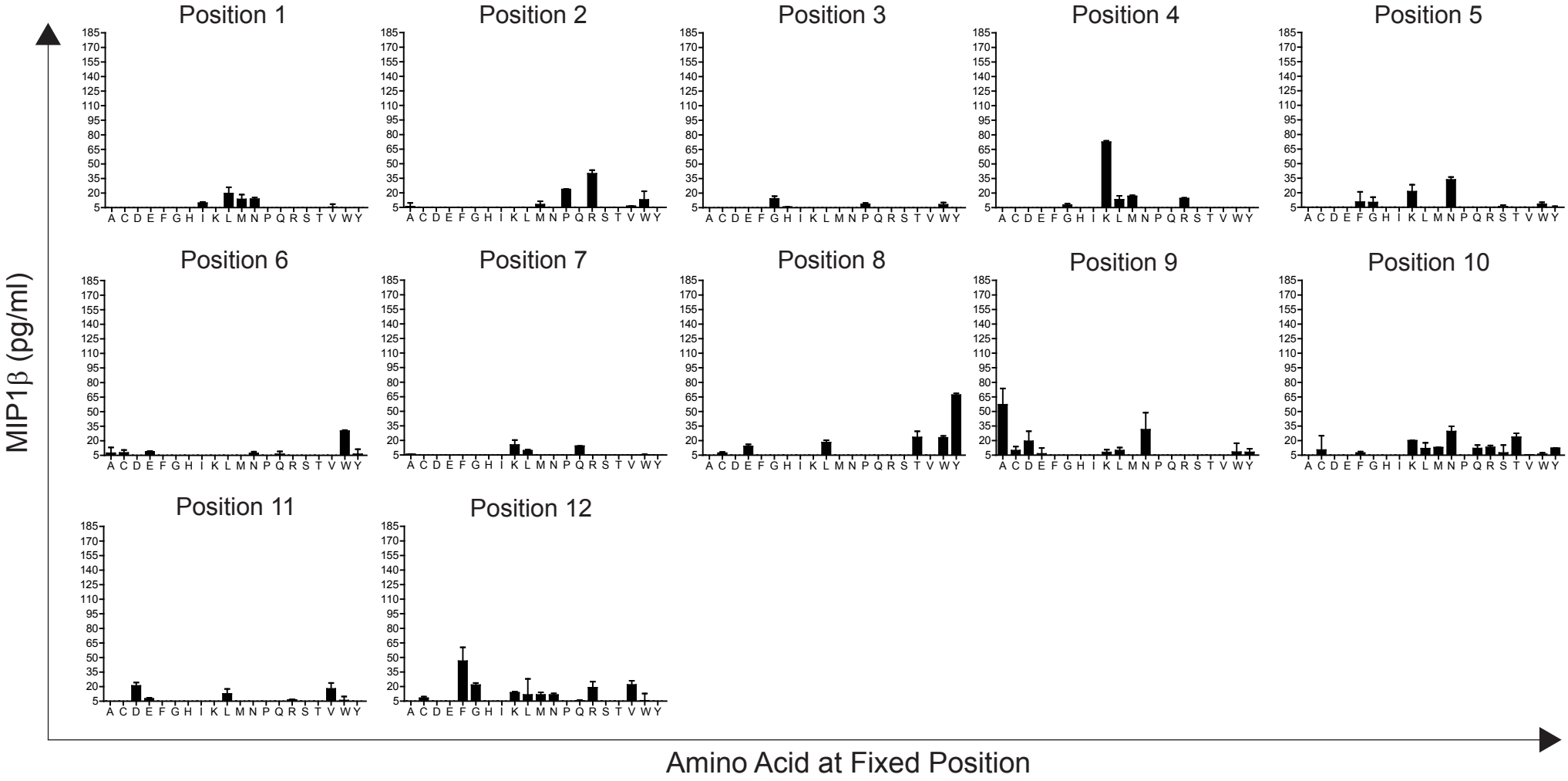
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid at Fixed Position

Figure S2E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Position 4
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Position 6
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Position 8
Position 9
Position 10
Position 11
Position 12
MIP1β (pg/ml)
Amino Acid at Fixed Position
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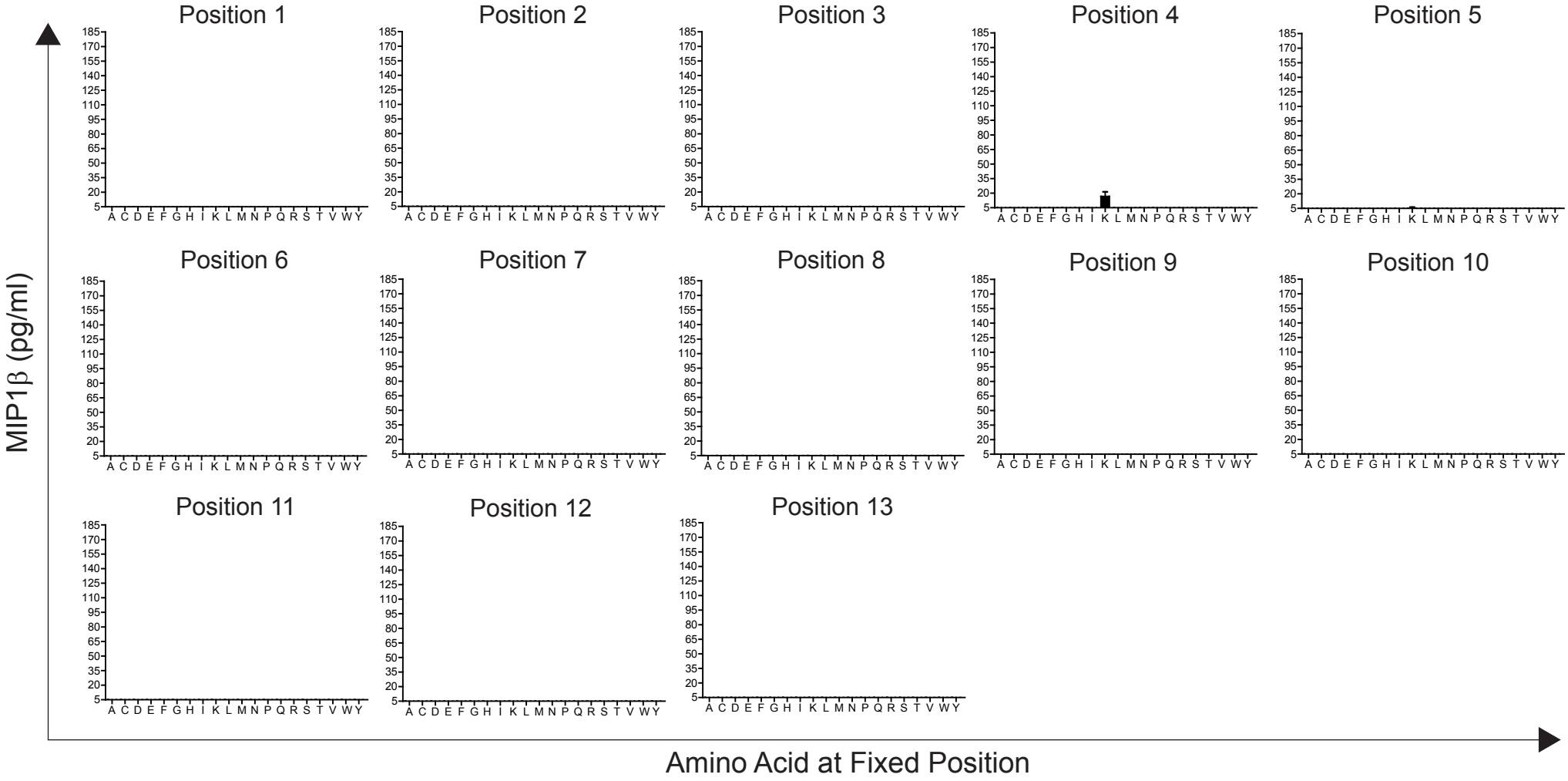
185 170 155 140 125 110 95 80 65 50 35 20 5

Figure S2F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Position 8
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Position 10
Position 11
Position 12
Position 13
MIP1β (pg/ml)
Amino Acid at Fixed Position
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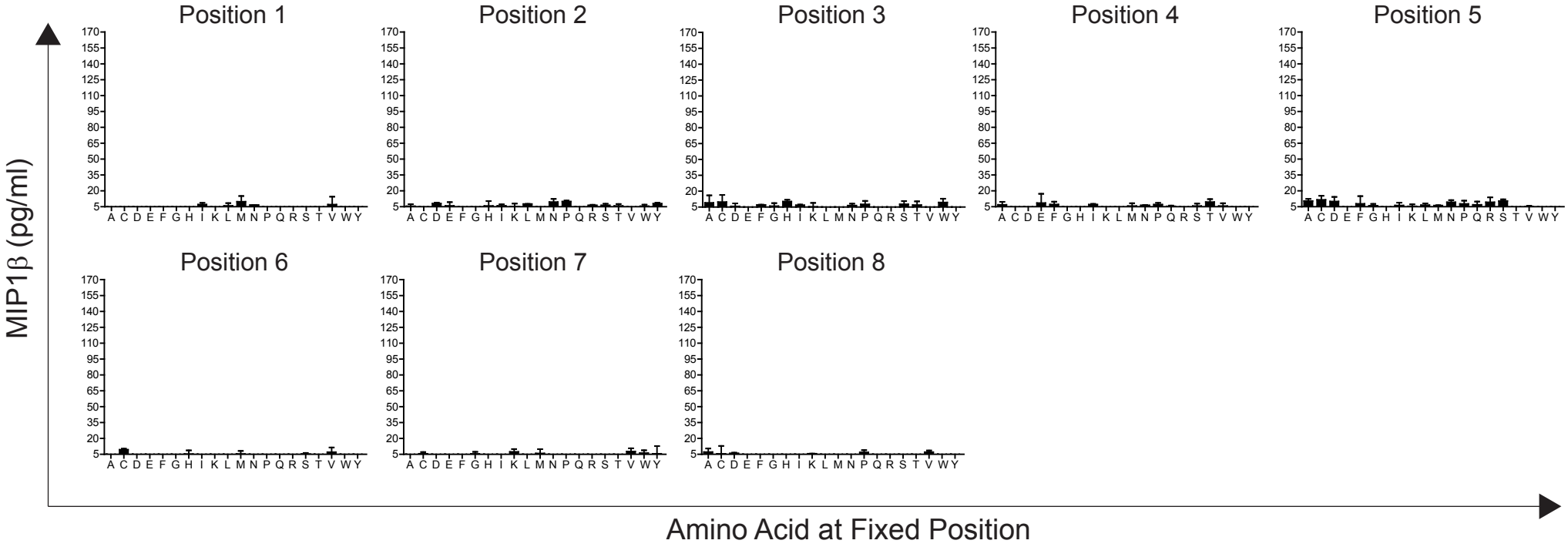
185 170 155 140 125 110 95 80 65 50 35 20 5

Figure S3A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Position 2
Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
MIP1β (pg/ml)
170
155
140
125
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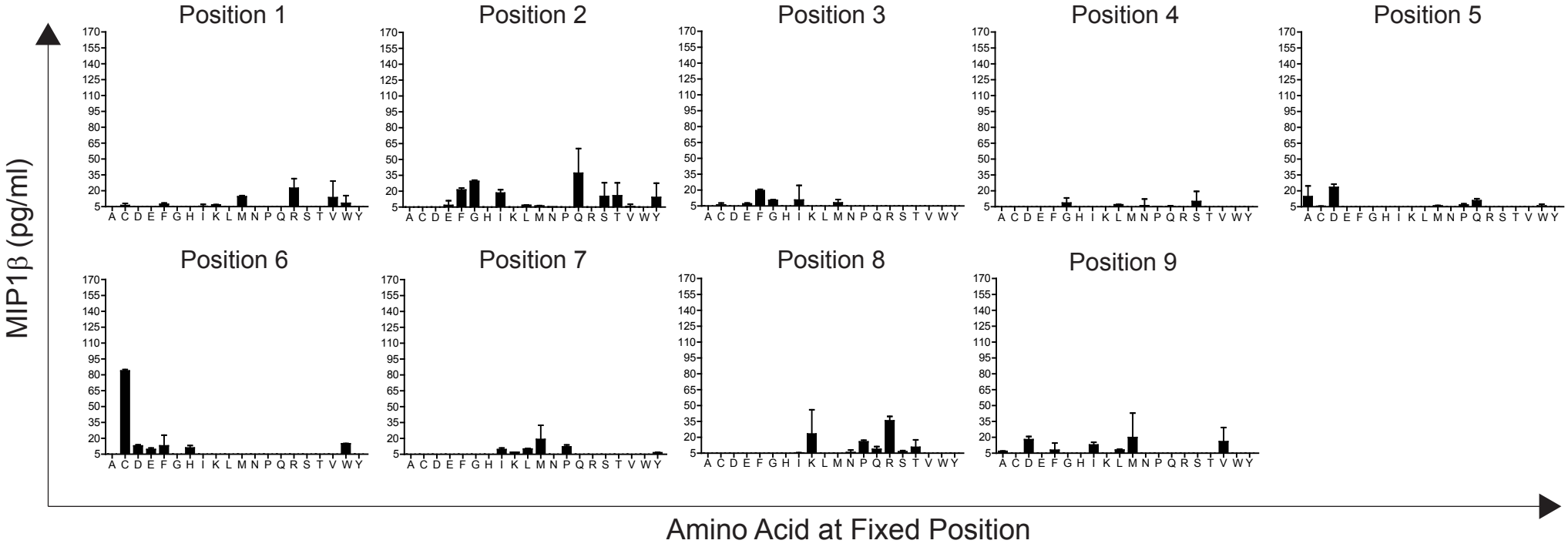
95
80
65
50
35
20
5
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid at Fixed Position

Figure S3B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Position 4
Position 5
Position 6
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Position 8
Position 9
MIP1β (pg/ml)
Amino Acid at Fixed Position
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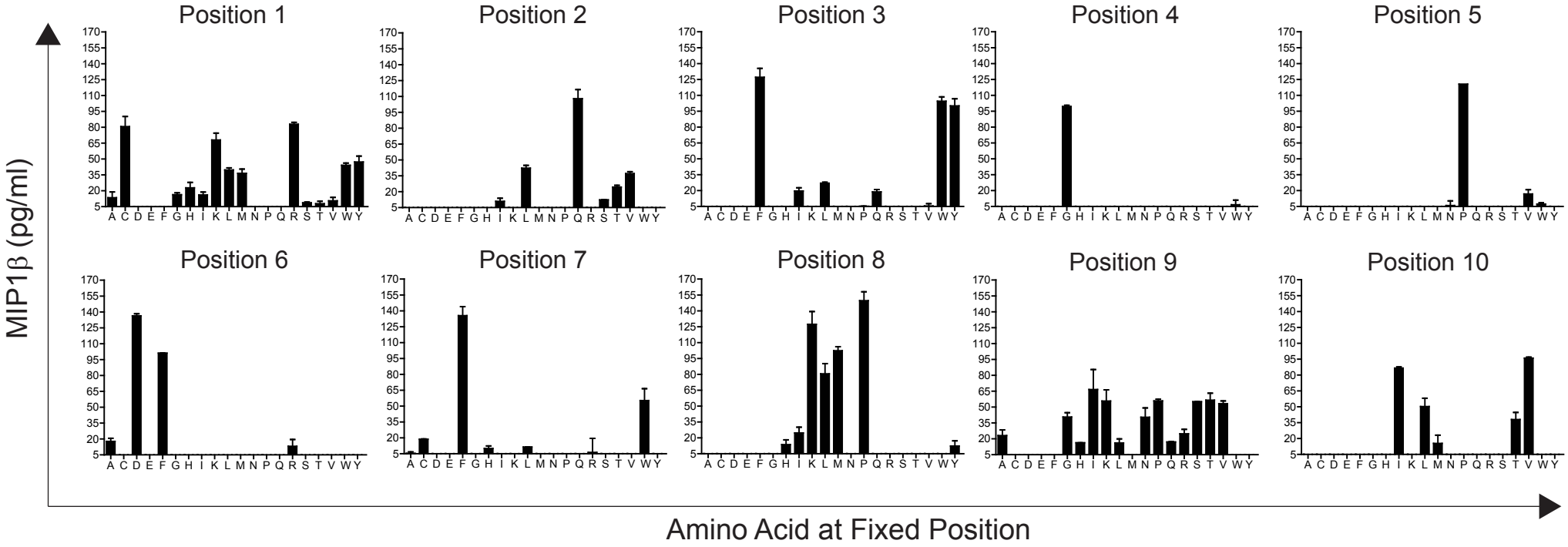
Figure S3C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Position 4
Position 5
Position 6
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MIP1β (pg/ml)
Amino Acid at Fixed Position
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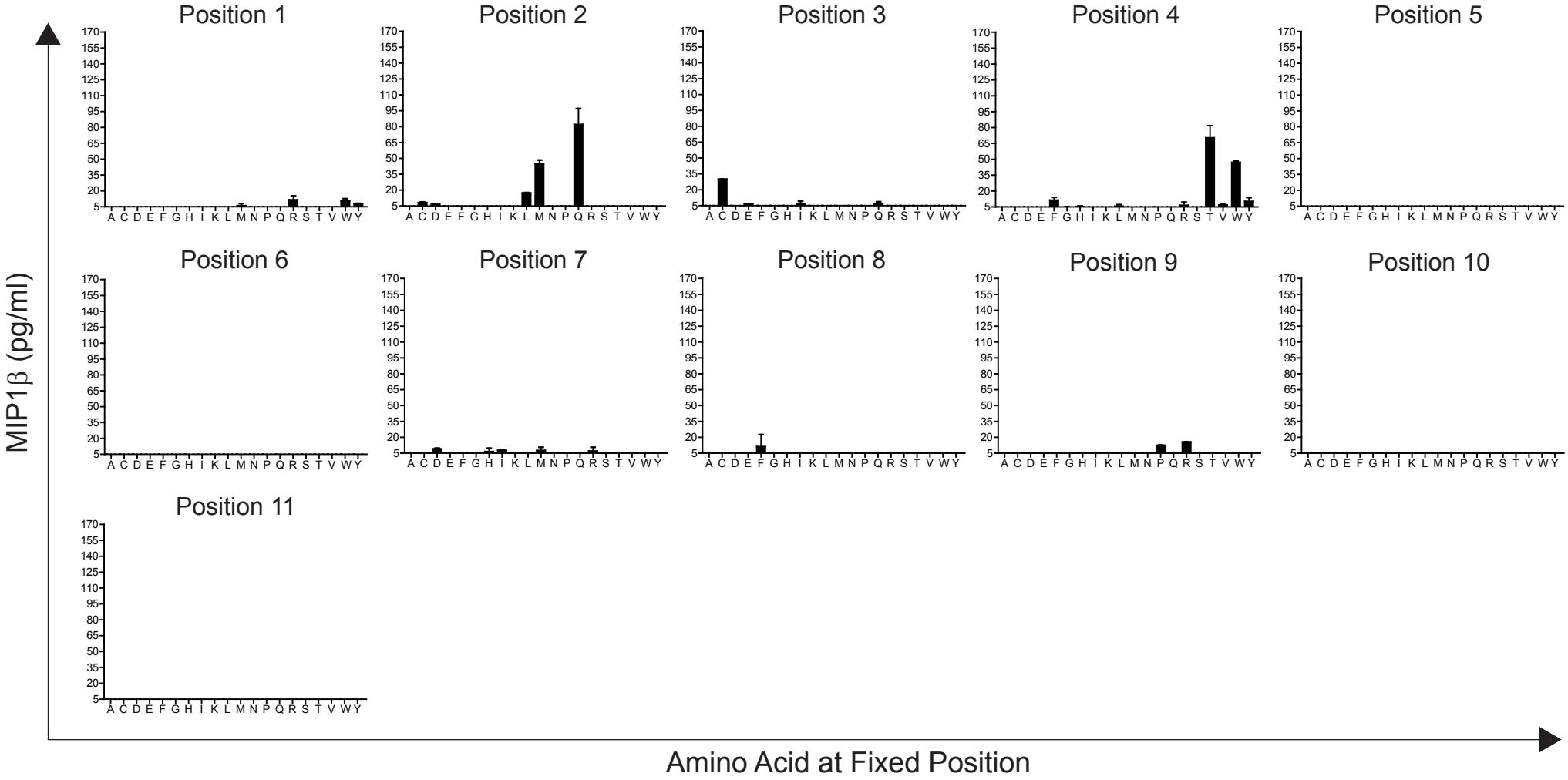
Figure S3D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Position 6
Position 7
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MIP1β (pg/ml)
Amino Acid at Fixed Position
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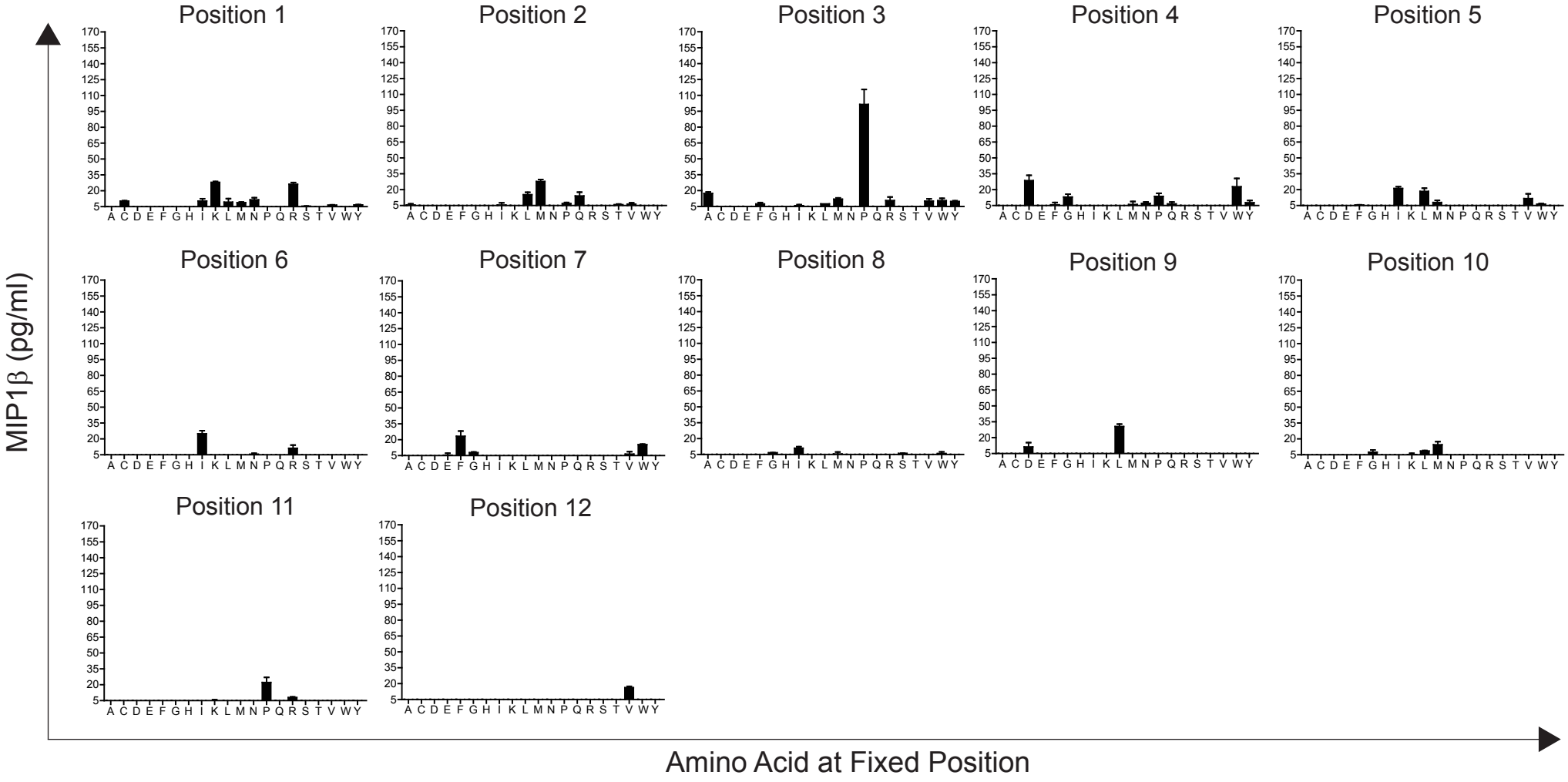
Figure S3E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Position 9
Position 10
Position 11
Position 12
MIP1β (pg/ml)
Amino Acid at Fixed Position
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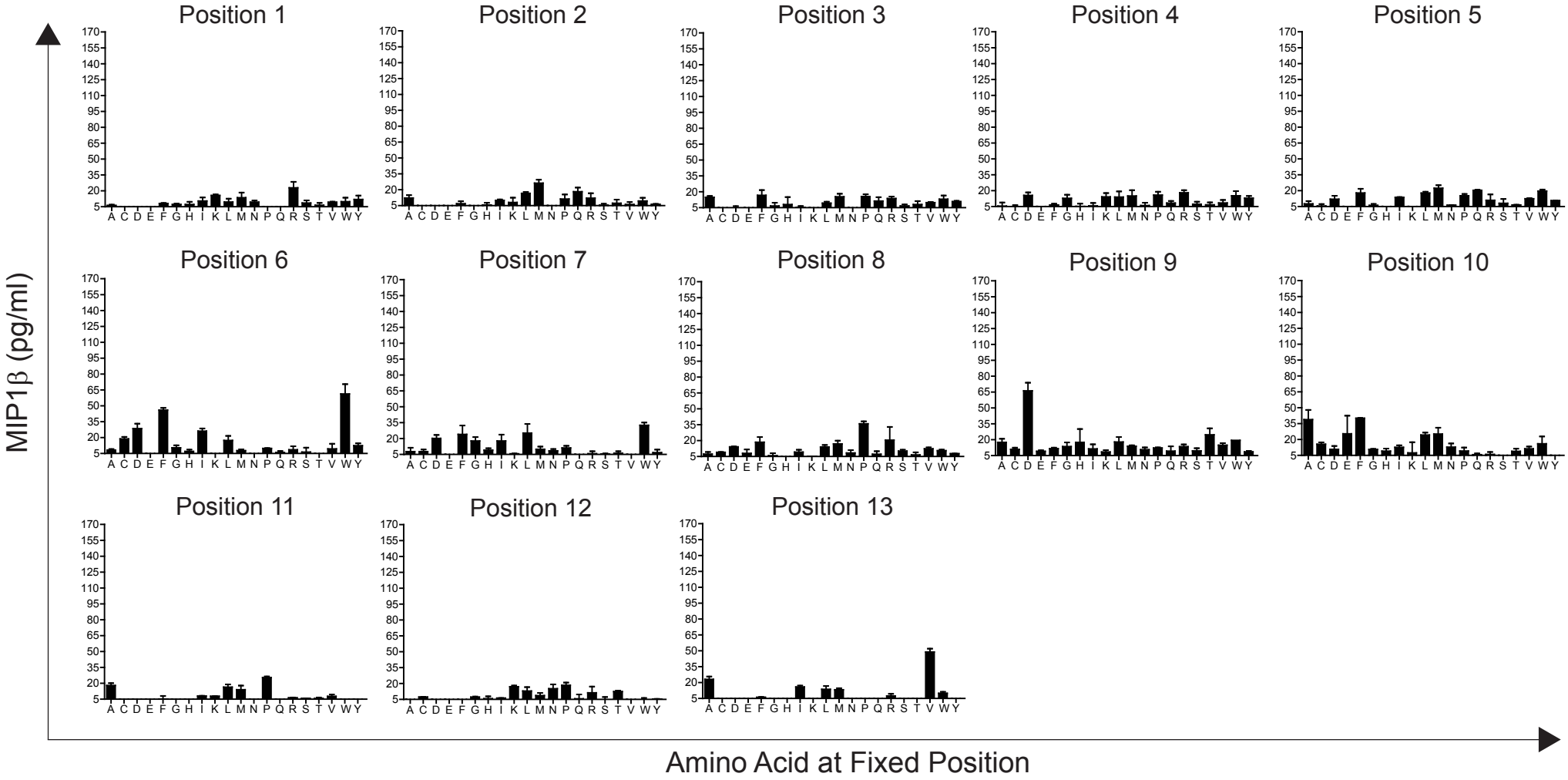
Figure S3F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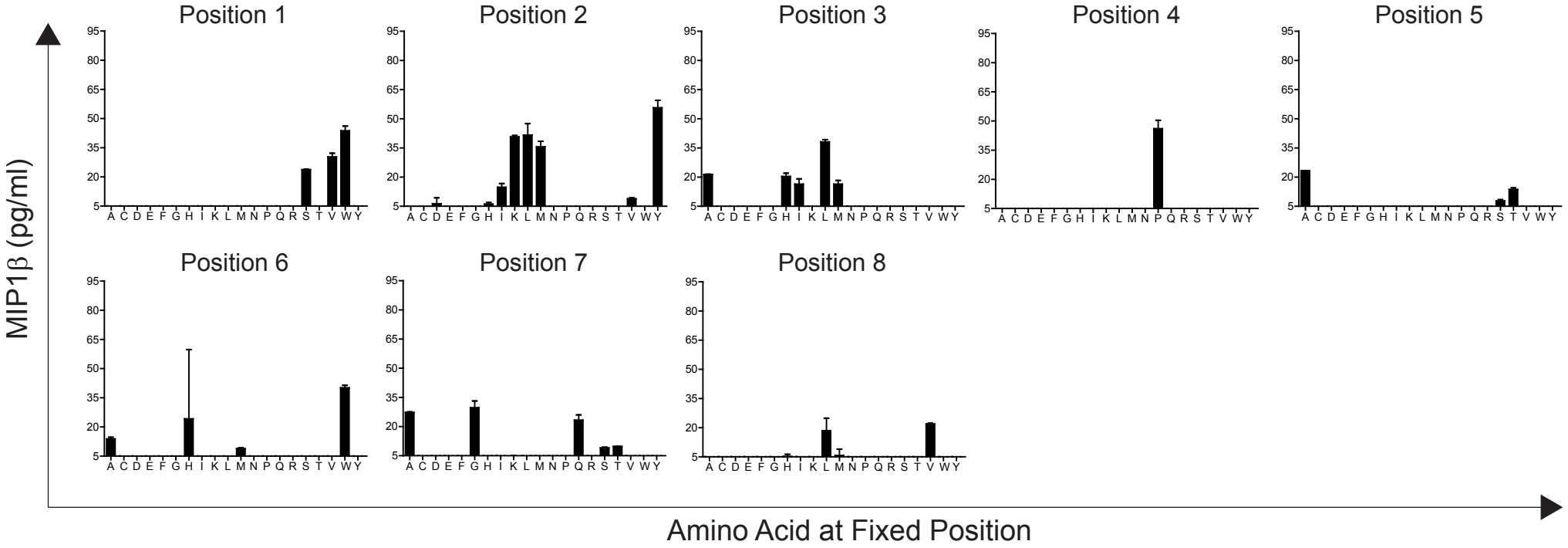
MIP1β (pg/ml)
Amino Acid at Fixed Position
A C D E F G H I K L M N P Q R S T V W Y

Figure S4A


Position 1
Position 2
Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
MIP1β (pg/ml)
95
80
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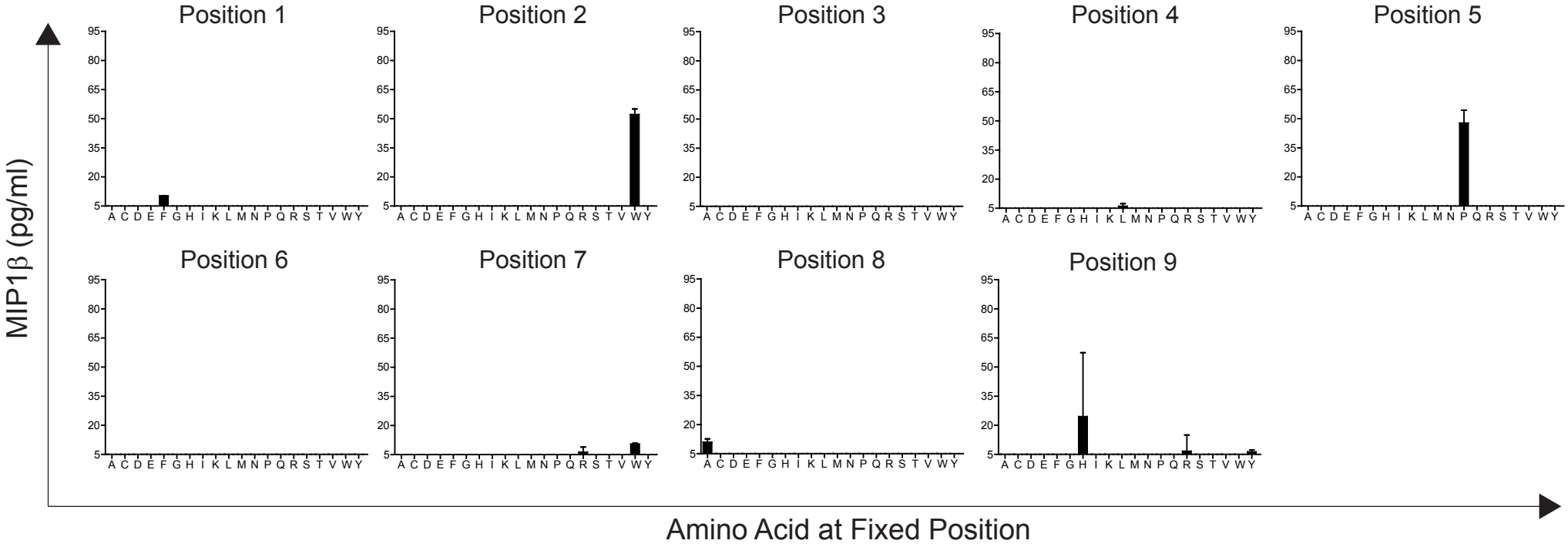
20
5
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid at Fixed Position

Figure S4B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Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
Position 9
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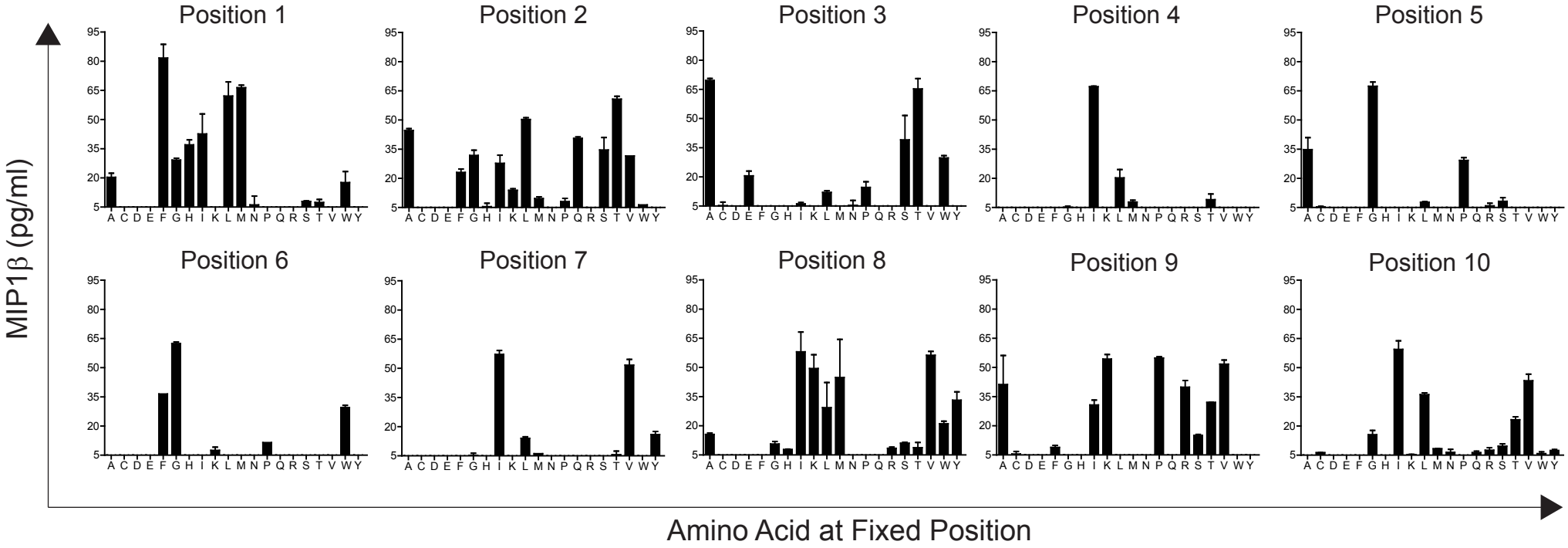
MIP1β (pg/ml)
Amino Acid at Fixed Position

Figure S4C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Position 5
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Position 8
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Position 10
MIP1β (pg/ml)
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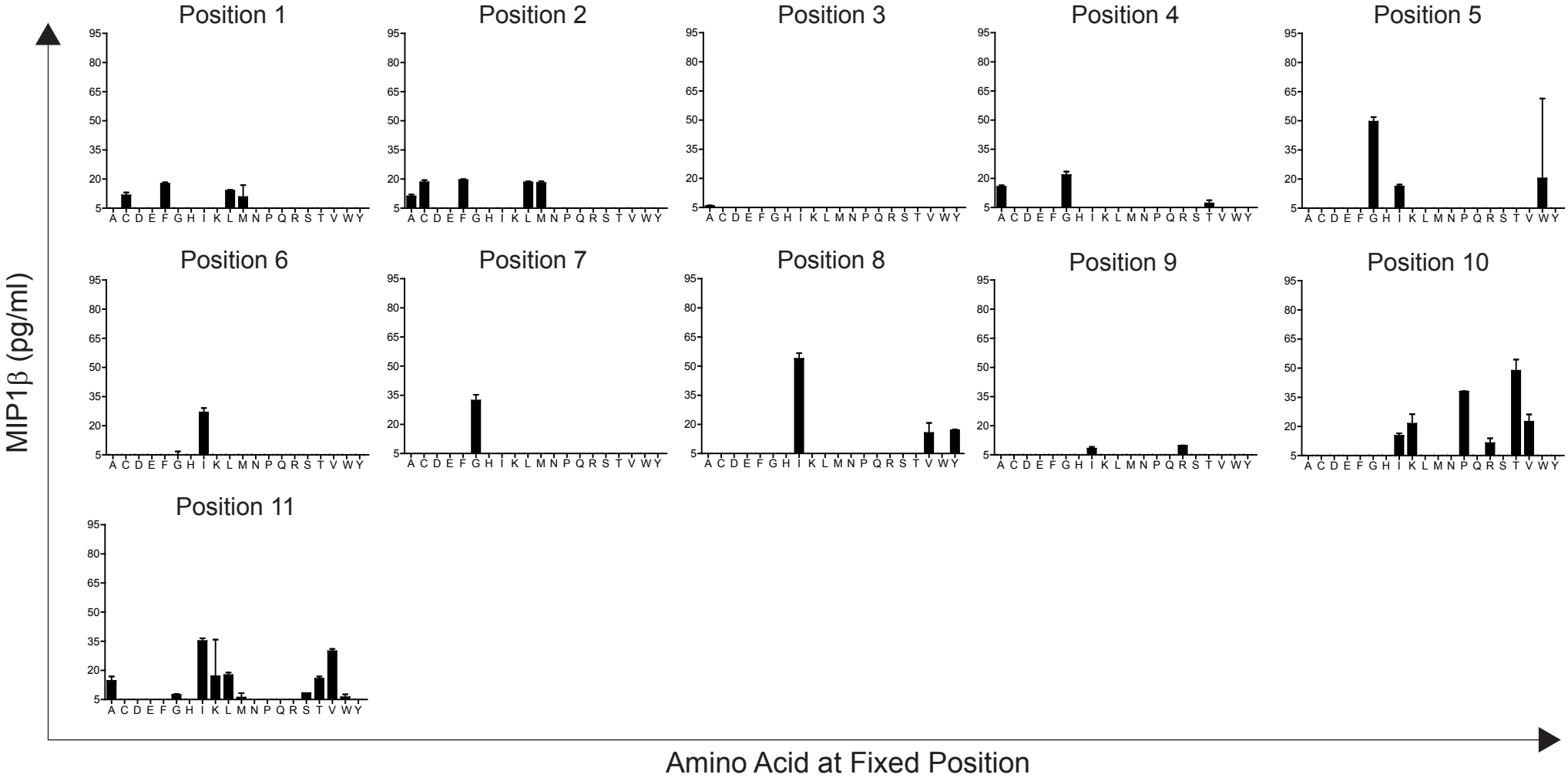
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid at Fixed Position

Figure S4D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Position 5
Position 6
Position 7
Position 8
Position 9
Position 10
Position 11
MIP1β (pg/ml)
Amino Acid at Fixed Position
95
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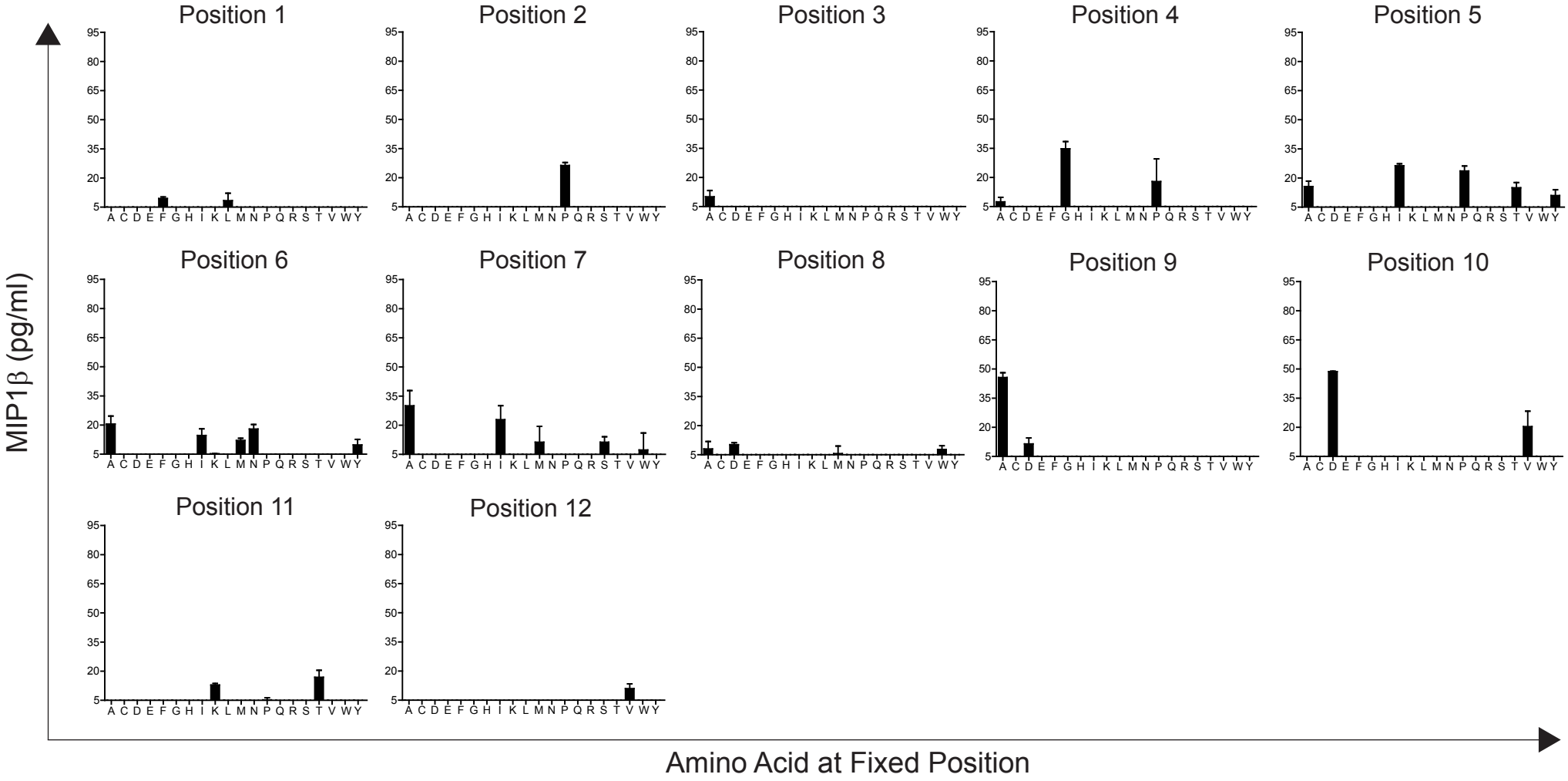
A C D E F G H I K L M N P Q R S T V W Y

Figure S4E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Position 7
Position 8
Position 9
Position 10
Position 11
Position 12
MIP1β (pg/ml)
Amino Acid at Fixed Position
95
80
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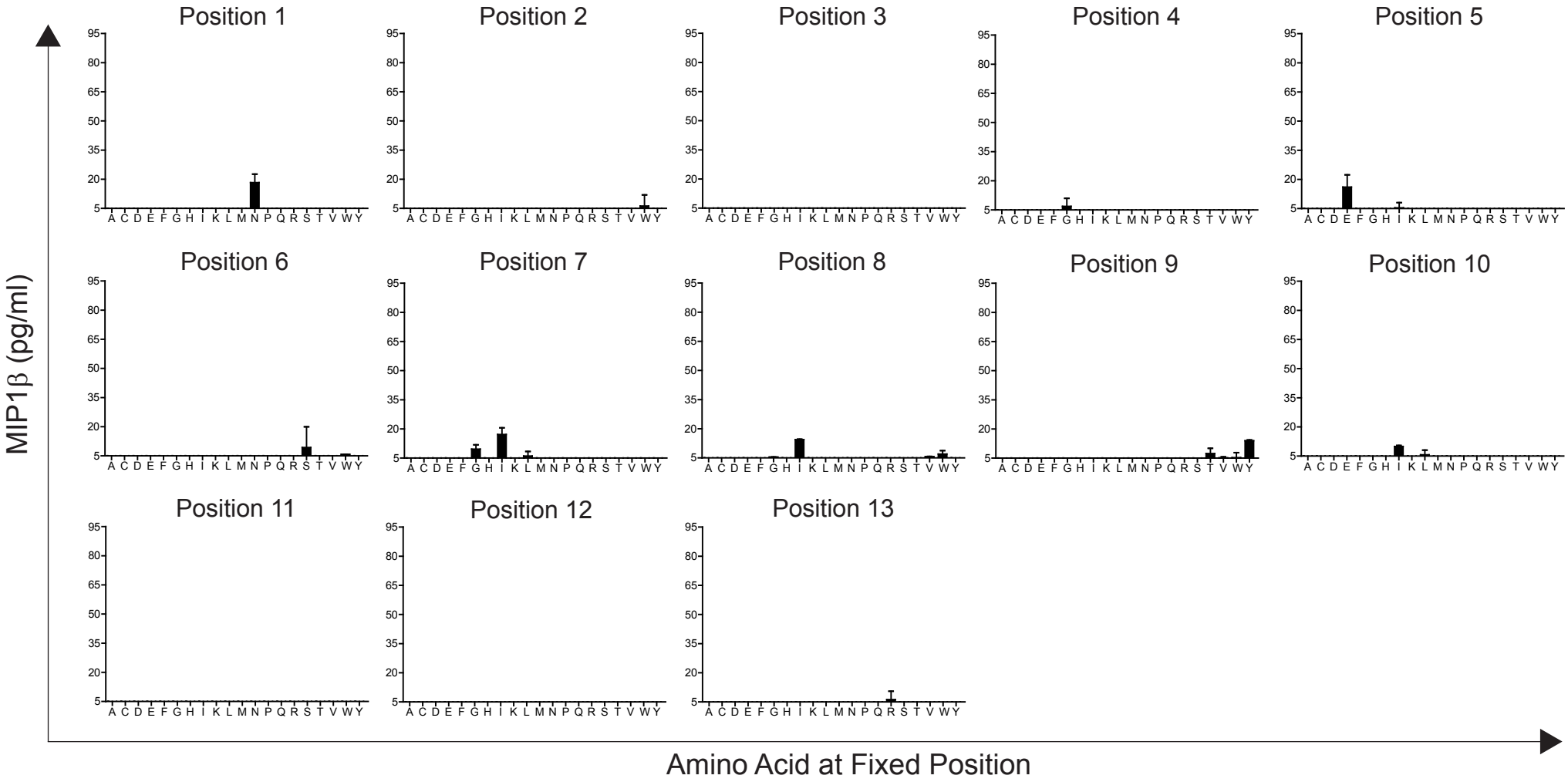
A C D E F G H I K L M N P Q R S T V W Y

Figure S4F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Position 13
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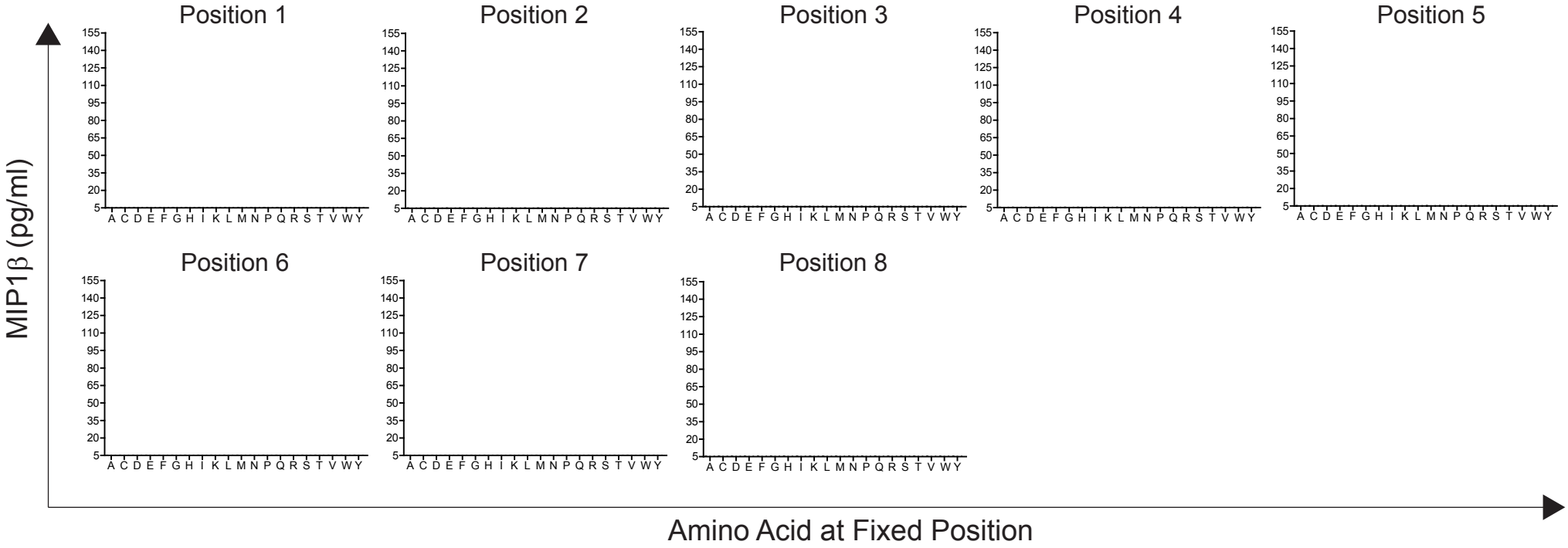
A C D E F G H I K L M N P Q R S T V W Y
MIP1β (pg/ml)
Amino Acid at Fixed Position

Figure S5A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Position 2
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Position 8
155
140
125
110
95
80
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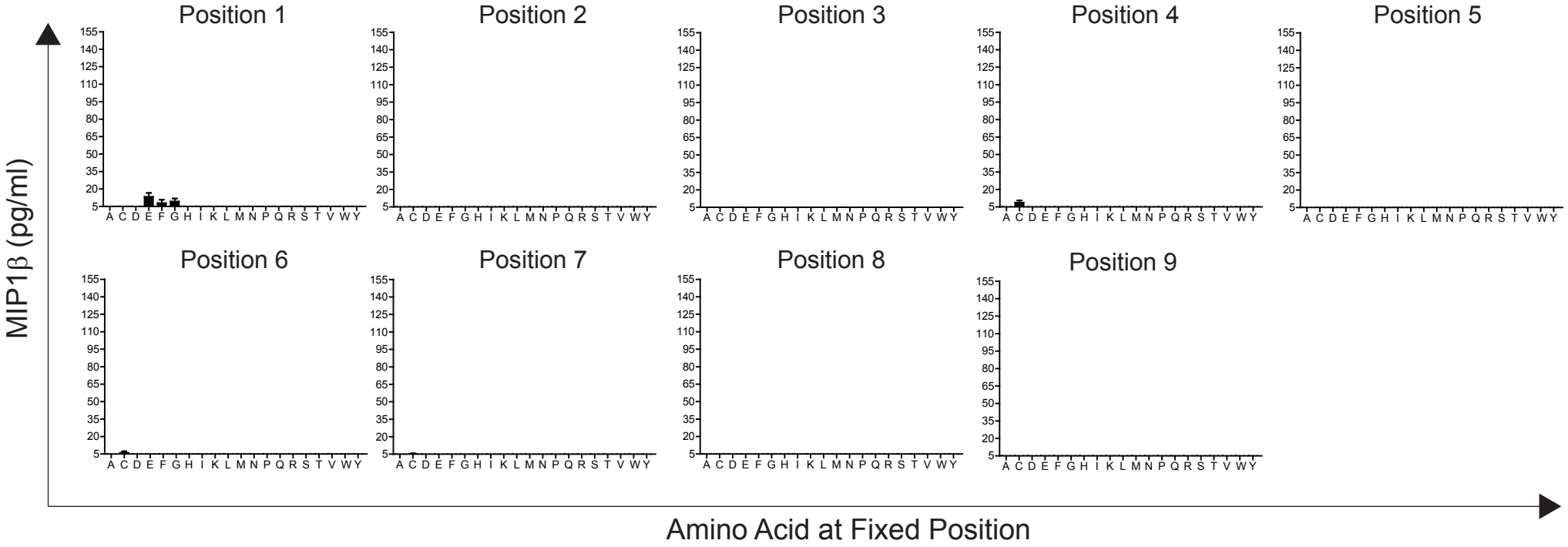
5
A C D E F G H I K L M N P Q R S T V W Y
MIP1β (pg/ml)
Amino Acid at Fixed Position

Figure S5B


Position 1
Position 2
Position 3
Position 4
Position 5
Position 6
Position 7
Position 8
Position 9
MIP1β (pg/ml)
155
140
125
110
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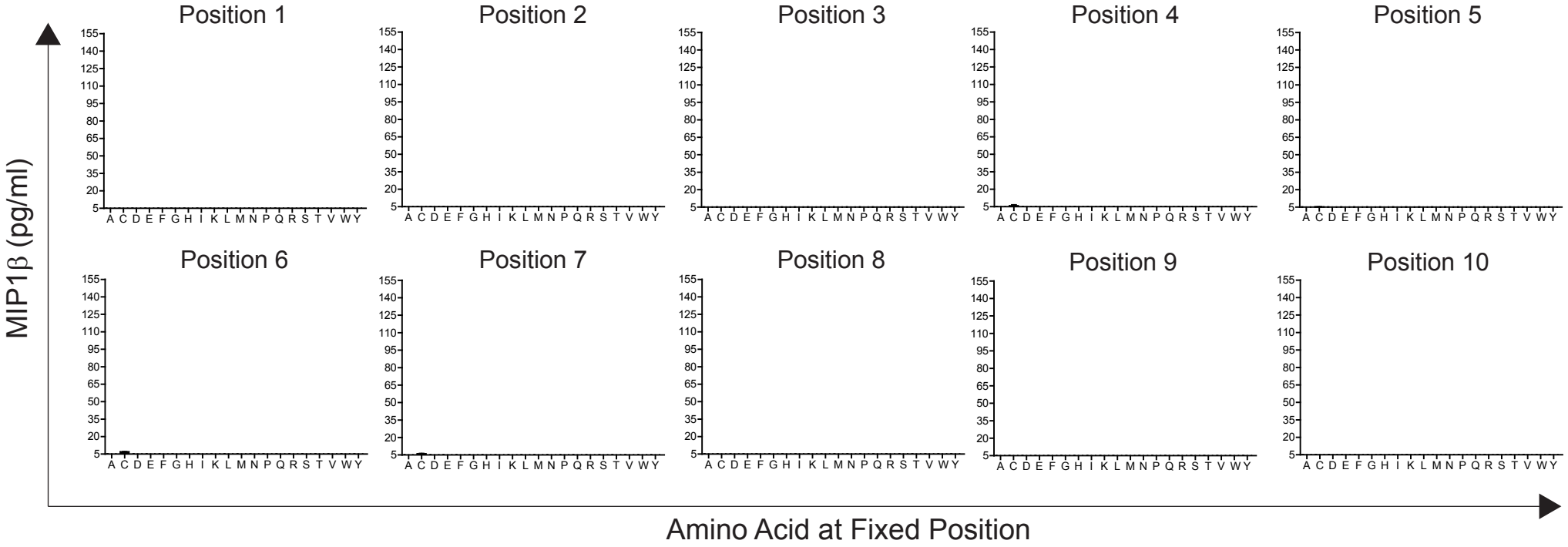
20
5
A C D E F G H I K L M N P Q R S T V W Y
Amino Acid at Fixed Position

Figure S5C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Position 10
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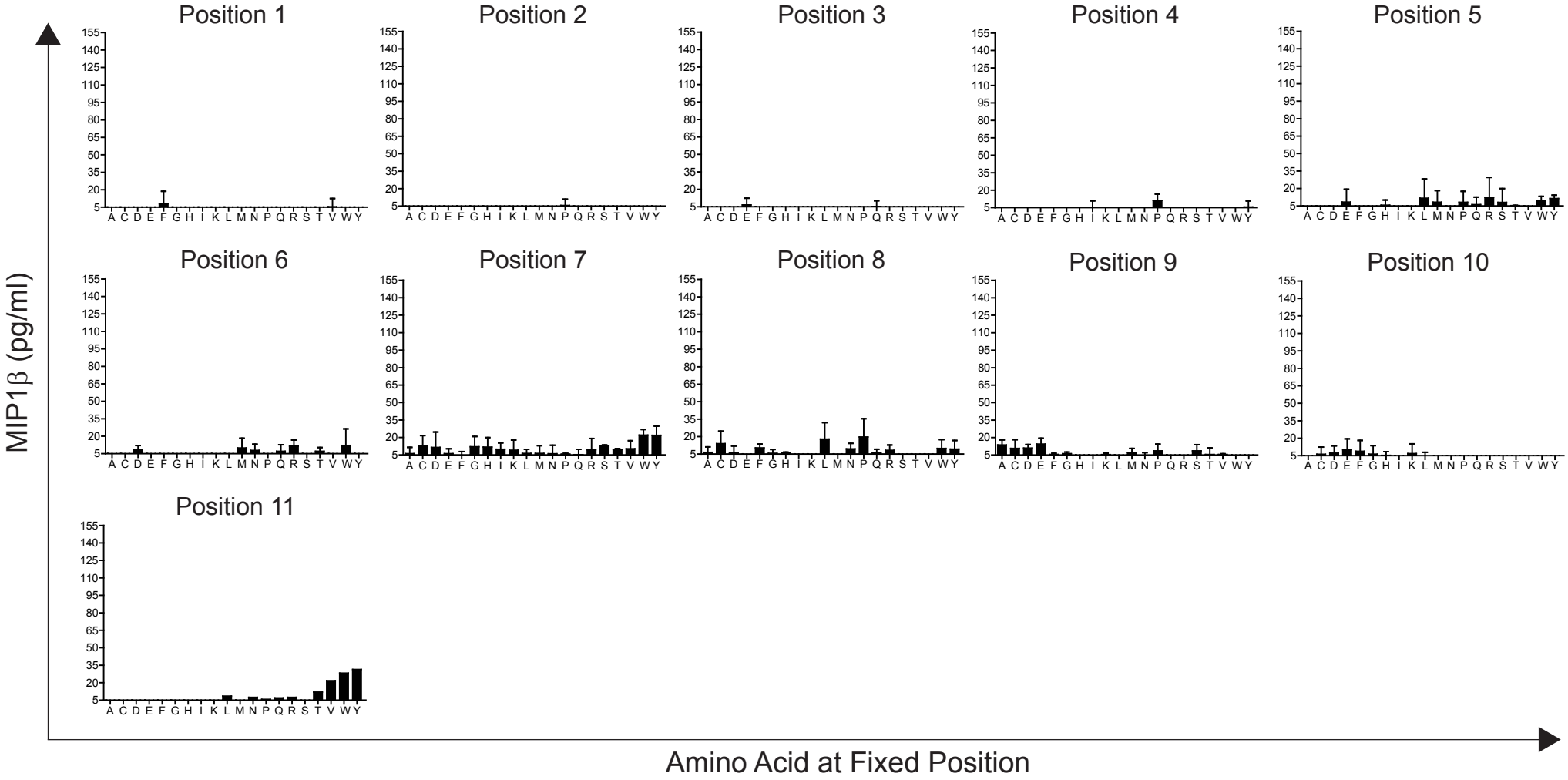
Amino Acid at Fixed Position

Figure S5D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MIP1β (pg/ml)
Amino Acid at Fixed Position
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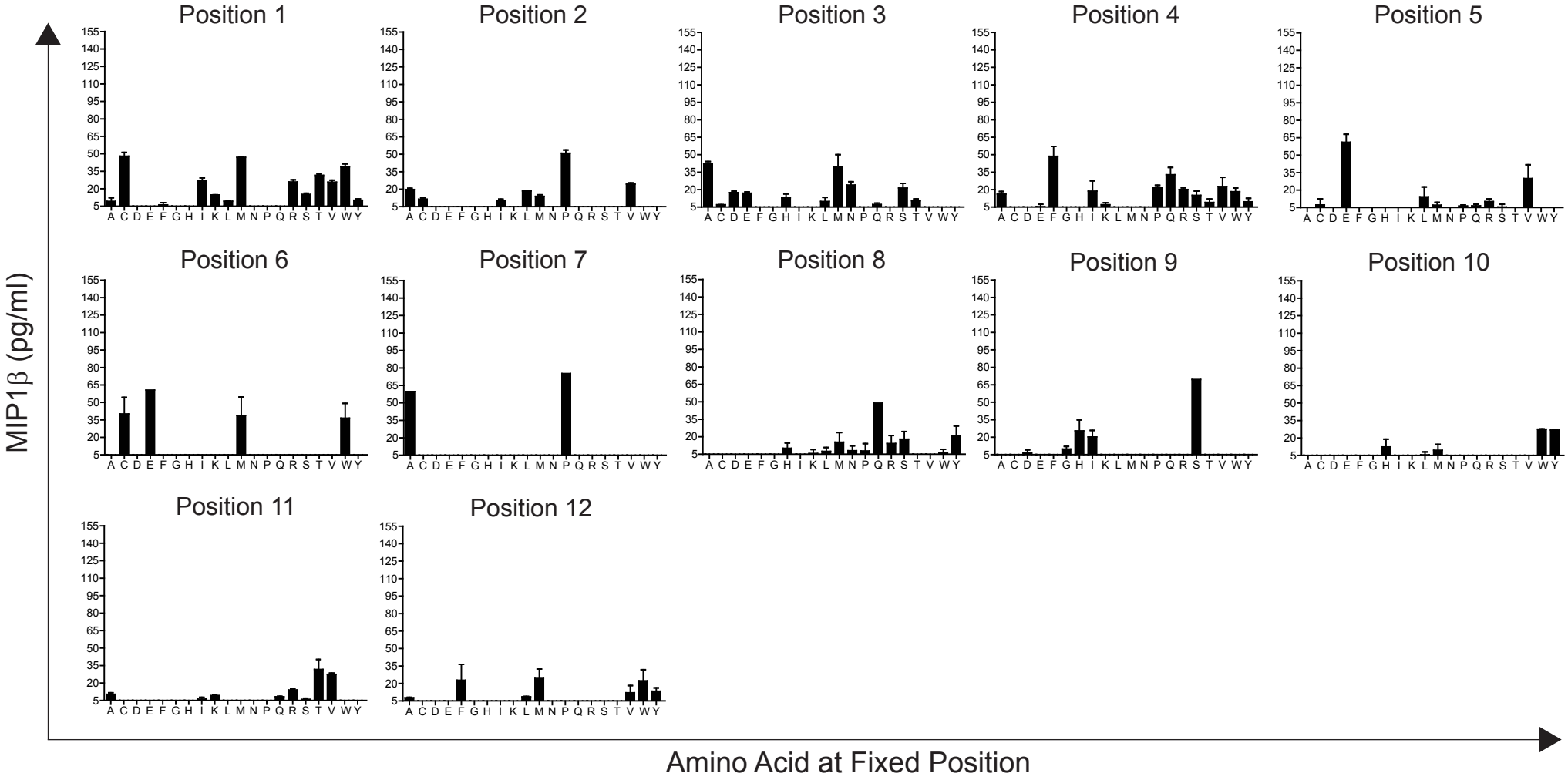
Figure S5E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Amino Acid at Fixed Position
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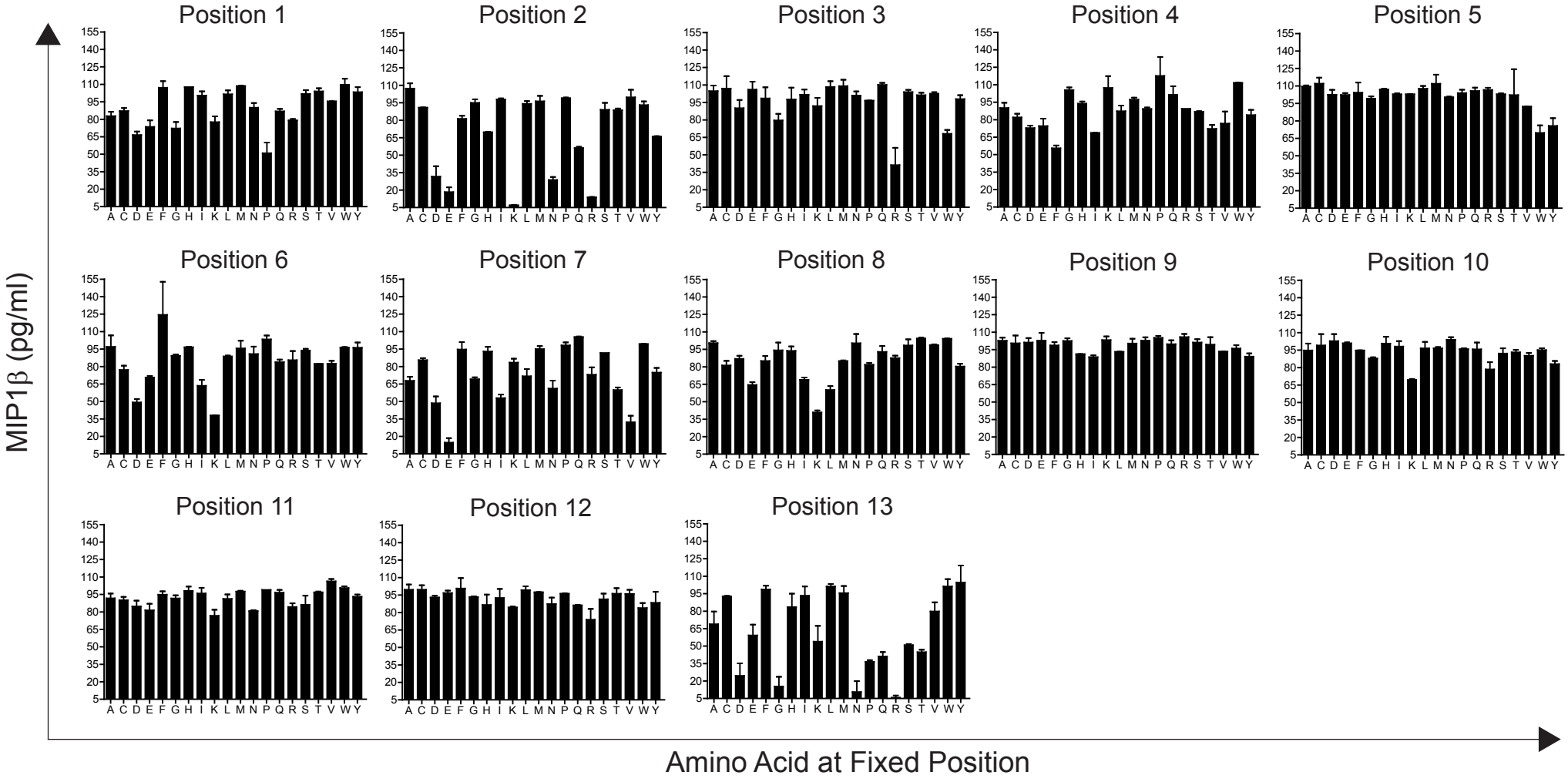
Figure S5F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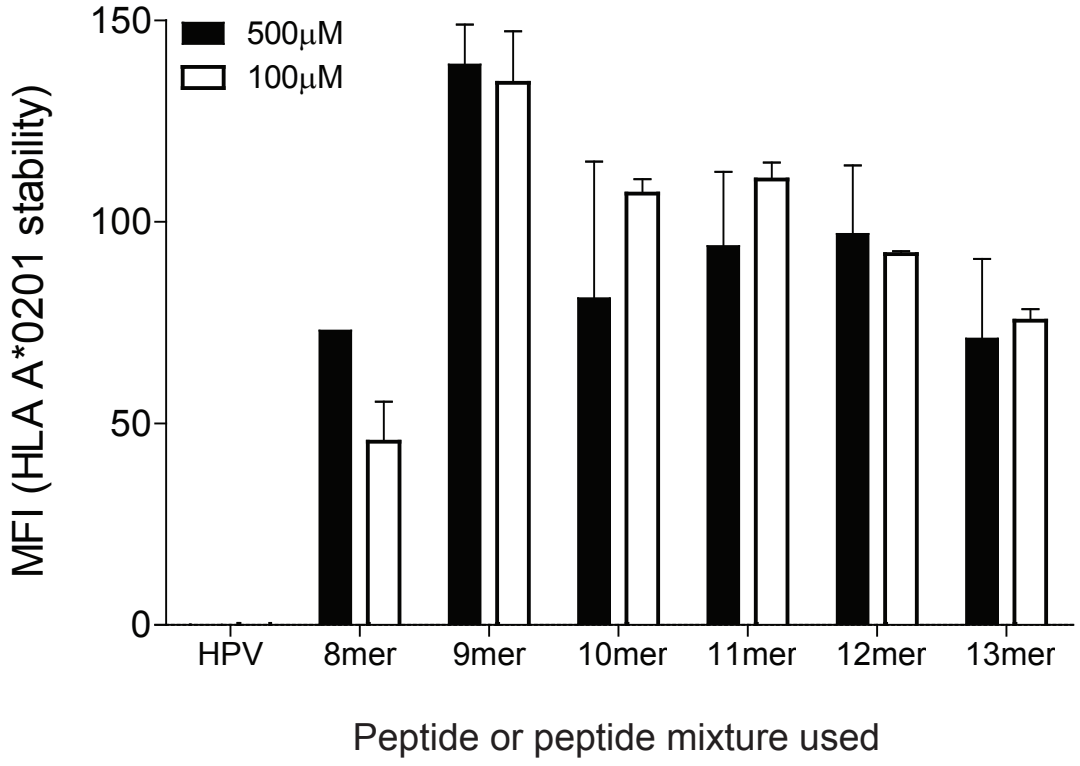
Position 13
MIP1β (pg/ml)
Amino Acid at Fixed Position

A: Standard Protocol



B: Melting Protocol

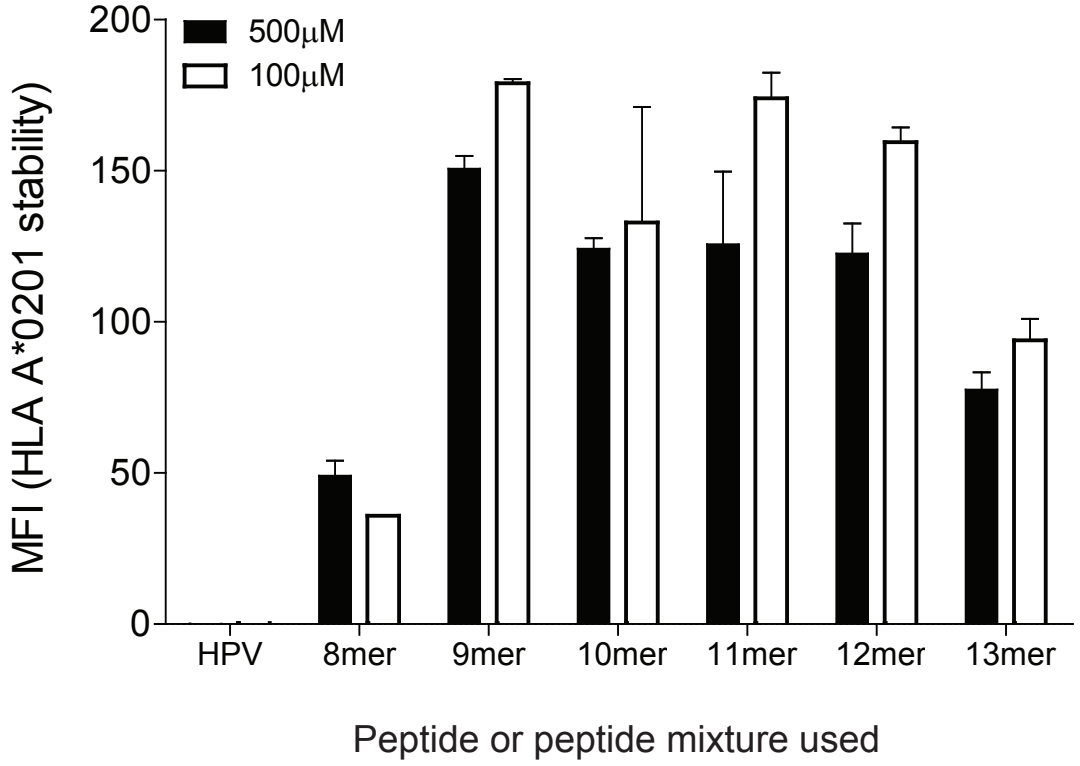
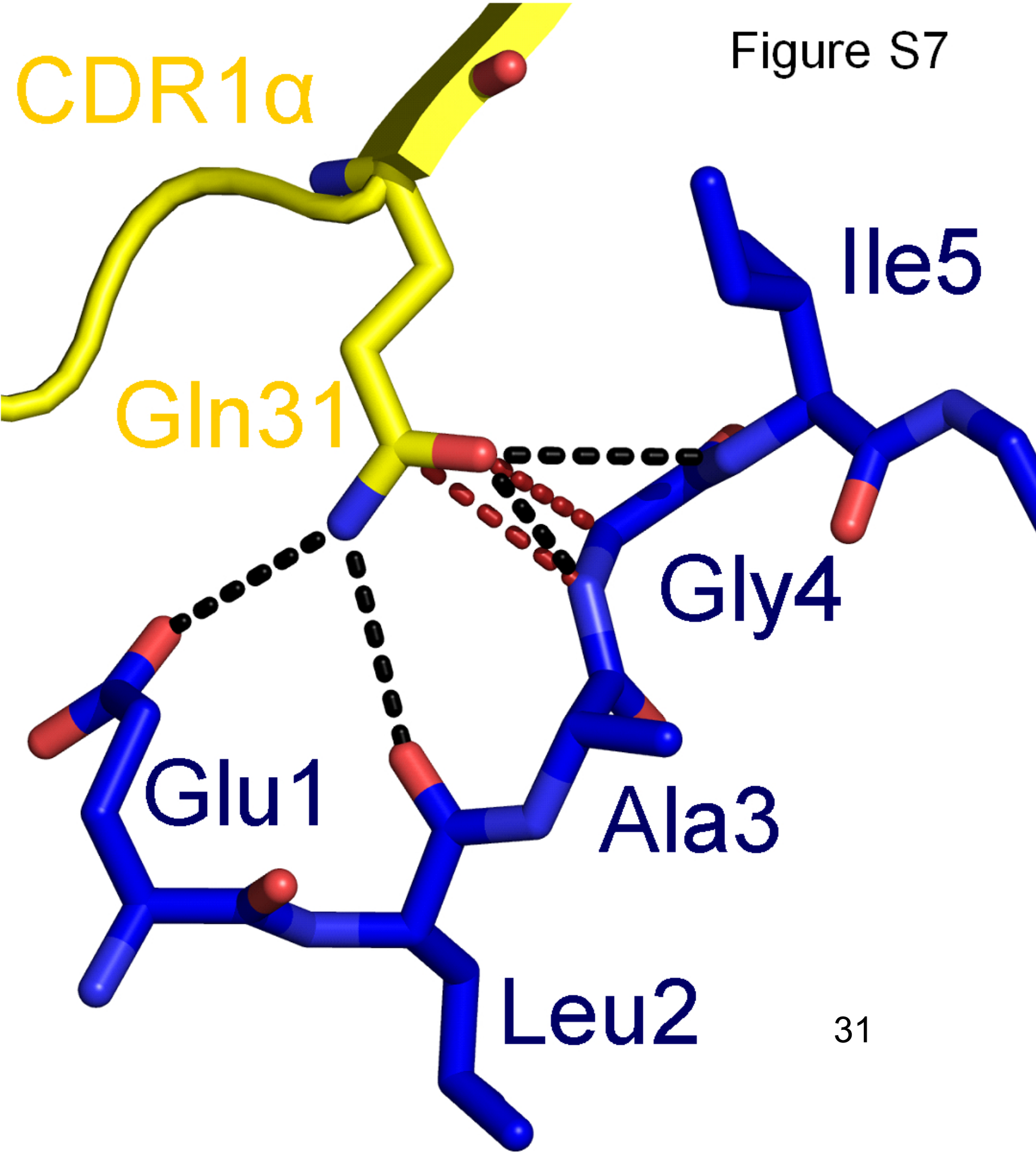


Figure S7



A: 11mer biased sampling

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2	YQITPPIFLKR	-4.076353031
3	RDETGYRFPTG	-4.255877311
4	RQEWPPMFPIG	-4.661464173
5	WQETGPMFRNF	-4.688921303
6	YQEWGDIWPLG	-4.996010737
7	WQITPPDDPIG	-5.020000311
8	NIITGPHFRII	-5.102310623
9	WQITGPMIRIG	-5.681094549
10	YMQTTDRFRIG	-5.797596935
11	YDIWGPIFRII	-5.849335338
12	VQETPPIFRHV	-5.896686297
13	MQQTGPDIRIG	-6.132221085
14	EQITGPIIPTG	-6.265725438
15	WMQWGPDFPFG	-6.446676225
16	KDIFSDIFRTI	-6.549390411
17	MDLTGPMFRKA	-6.906850047
18	YMQWTPDFPIG	-7.101066239
19	NMIFGSHFRLG	-105.9132494
20	TQFWPPIFPLI	-159.7550993
21	SEEWPPRIRIQ	-187.5793078
22	TLEWGPMFPKG	-214.42073
23	KQHTGDDFPKD	-367.0314757
24	SQITGPMFPRV	-821.1961667
25	NIEWPPRIRIF	-1151.492692
26	MQIVPDHFRIW	-1155.982093
27	RAFVGRDFRKG	-3224.023004
28	NRIVGDIFPKG	-6428.198759
29	SMIWPPIMRKG	-12817.2863
30	YMIWGDIFLNN	-25612.46014

B: 12mer biased sampling

Number	Peptide Sequence	pEC_{50}-$pEC_{50\ (index)}$
Index	ALWGPDPAAA	0
1	MMLWLRWIDVPT	-0.89411
2	KPADIIDALVPV	-3.37882
3	VVAPIWESLSNV	-3.86078
4	KQRPWIGDLMQG	-4.03667
5	RQSHIAQLLGMV	-4.1435
6	RQVNIWWLDMQL	-4.15599
7	KPFDWNPFLMRT	-4.16008
8	KWPGIIWSLSPL	-4.21619
9	RLPGVPGILGRM	-4.29965
10	KPVPNNTWGTLW	-4.3645
11	RLWGIAFGDMKL	-4.40015
12	RMPTINGILTKM	-4.40383
13	IARLPTVDSTAV	-4.43509
14	LLYGIDDSLLGL	-4.46247
15	KLQEVIFILMML	-4.49806
16	RQWPLIVGDDML	-4.50341
17	LQVAVNGWLMQM	-4.53334
18	IMQFVRDISLPT	-4.61329
19	RLLYIIFLGEVG	-4.62168
20	RMTGIIGDDLQG	-4.657
21	RVMGLDFMGPQV	-4.6655
22	RMPMLIWWAMLV	-4.67491
23	KIPGINMDLVIV	-4.71077
24	MLPPVIFWLTEV	-4.83171
25	RVPDIATAEVNV	-4.96712
26	IQPPVRGIDMPV	-5.11124
27	MLMDIDWSMGPM	-5.14999
28	YQPLIRVPLDPM	-5.51261
29	GTWMIIFIWSPV	-212724
30	LIPDNNFIAEPS	-425432

Table S2 Data collection and refinement statistics (molecular replacement).

Data set statistics	HLA A*0201-ILAKFLHRL
Space group	$P2_1\,2_1\,2_1$
Unit cell parameters (Å,°)	a=49.2, b=74.9, c=125.8
Radiation source	DIAMOND I03
Wavelength (Å)	0.9763
Resolution (Å)	49.2 - 1.78
Unique reflections	45414 (3326)
Completeness (%)	100
Multiplicity	7.1 (6.5)
I/Sigma(I)	10 (2.4)
Rmerge (%)	0.109 (0.677)
No reflections used	43,626
Rcryst (no cutoff) (%)	18
Rfree (%)	22.7
Bond lengths (Å)	0.022
Bond angles (°)	1.845
Mean B value ($Å^2$)	26.3
Outliers Ramachandran plot (%)	0
Overall ESU based on Maximum Likelihood (Å)	0.082

One crystal was used for data collection.
Values in parentheses are for highest-resolution shell.