

SUPPLEMENTARY MATERIAL TO:

Research Article

Component-resolved diagnosis based on a recombinant variant of Mus m 1 lipocalin allergen

Elena Ferrari ^{1*}, Daniela Breda ², Alberto Spisni ¹ and Samuele E. Burastero ²

¹ Department of Medicine and Surgery, University of Parma, Parma, Italy

² Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

* Correspondence: elena.ferrari@unipr.it

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CLUSTAL O(1.2.4) multiple sequence alignment

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EMBOSS_001          -----EASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      41
sp|P11589|MUP2_MOUSE -MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      59
sp|P04938|MUP11_MOUSE MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      60
sp|P11588|MUP1_MOUSE  -MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      59
tr|A9R9W0|A9R9W0_MOUSE -MKMLLLLCLGLTLVCVHAEEASSTGRNFNQKINGEWHTIILASDKREKIEDNGNFRLF      59
tr|A2CEK7|A2CEK7_MOUSE -MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      59
tr|Q58EV3|Q58EV3_MOUSE -MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      59
tr|A2AKN8|A2AKN8_MOUSE -MKMLLLLCVGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNFRLF      59
sp|B5X0G2|MUP17_MOUSE -MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEEHGNFRLF      59
tr|A2CEK6|A2CEK6_MOUSE -MKMLLLLCLGLTLVCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEEHGNFRLF      59
                        *****:*****:*****

EMBOSS_001          LEQIHVLEKSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      101
sp|P11589|MUP2_MOUSE  LEQIHVLEKSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
sp|P04938|MUP11_MOUSE LEQIHVLENSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      120
sp|P11588|MUP1_MOUSE  LEQIHVLENSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
tr|A9R9W0|A9R9W0_MOUSE LEQIHVLENSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
tr|A2CEK7|A2CEK7_MOUSE LEQIRVLENSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
tr|Q58EV3|Q58EV3_MOUSE LEQIHVLENSLVLKVHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
tr|A2AKN8|A2AKN8_MOUSE LEQIRVLENSLVLKVHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
sp|B5X0G2|MUP17_MOUSE LEQIHVLENSLVLKVHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYDNFL      119
tr|A2CEK6|A2CEK6_MOUSE LEQIHVLENSLVLKVHTVRDEECSELSMVADKTEKAGKYSVTYDGFNTFTIPKTDYDNFL      119
                        ****.***.*****.******:*****:*****

EMBOSS_001          MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCLQAR      161
sp|P11589|MUP2_MOUSE  MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAKLCEEHGILRENIIDLSNANRCLQAR      179
sp|P04938|MUP11_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCLQAR      180
sp|P11588|MUP1_MOUSE  MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEKHGILRENIIDLSNANRCLQAR      179
tr|A9R9W0|A9R9W0_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEKHGILRENIIDLSNANRCLQAR      179
tr|A2CEK7|A2CEK7_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCLQAR      179
tr|Q58EV3|Q58EV3_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEKHGILRENIIDLSNANRCLQAR      179
tr|A2AKN8|A2AKN8_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCLQAR      179
sp|B5X0G2|MUP17_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCLQAR      179
tr|A2CEK6|A2CEK6_MOUSE MAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCLQAR      179
                        *****:***:*****

EMBOSS_001          E*  162
sp|P11589|MUP2_MOUSE  E-  180
sp|P04938|MUP11_MOUSE E-  181
sp|P11588|MUP1_MOUSE  E-  180
tr|A9R9W0|A9R9W0_MOUSE E-  180
tr|A2CEK7|A2CEK7_MOUSE E-  180
tr|Q58EV3|Q58EV3_MOUSE E-  180
tr|A2AKN8|A2AKN8_MOUSE E-  180
sp|B5X0G2|MUP17_MOUSE E-  180
tr|A2CEK6|A2CEK6_MOUSE E-  180
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Figure S1. Amino acid sequence alignment of *central* mouse Major Urinary Proteins. Align tool, running the Clustal Omega algorithm to find sections of identity/similarity, was used for multiple sequence alignment. Consensus symbols: asterisk indicates a position which has a fully conserved residue; colon indicates a position which has a substitution between amino acids with similar properties (roughly equivalent to scoring > 0.5 in the Gonnet PAM 250 matrix [27]); period indicates a position which has almost one substitution between amino acids of weakly similar properties (roughly equivalent to scoring ≤ 0.5 and >0 in the Gonnet PAM 250 matrix). Each protein is represented by its accession number in UniProtKB database, while the query protein Mus m 1.0102 is indicated as EMBOSS_001 (dashed line at the beginning of the sequence indicates omission of the secretion signal).

CLUSTAL O(1.2.4) multiple sequence alignment

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EMBOSS_001          -----EASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNF      38
sp|Q5FW60|MUP20_MOUSE ---MKLLVLLLCGLTLVLCVHAEEASSMERNFNVEKINGEWYTIIMLATDKREKIEEHGSM      57
tr|A2AV72|A2AV72_MOUSE ----MKLLLLLCGLTLVLCVHAEEASSMGRNFNVEKINGEWYTIILASDKRAKIEEHGIM      55
sp|P11591|MUP5_MOUSE ----MKLLLLLCLELTLVYVHAEEASSEGNLNVEKINGKNFSILLASDKREKIEEHGTM      56
sp|P11590|MUP4_MOUSE ----MKLLLCGLTLVLCIHAEEATSKGQNLNVEKINGEWFISILLASDKREKIEEHGSM      54
tr|Q3KQQ2|Q3KQQ2_MOUSE MKLLLPLLLLLCLELTLVLCIHAEESSMERNFNVEQISGYWFSIAEASDEREKIEEHGSM      60
sp|P04939|MUP3_MOUSE MKLLLPLLLLLCLELTLVLCIHAEESSMERNFNVEQISGYWFSIAEASDEREKIEEHGSM      60
tr|Q80YX8|Q80YX8_MOUSE ---MKLLLLLCGLTIVLCIQAEEYSSMGRNFNVEQISGYWFSIAEASDEREKIEEHGSM      57
sp|P02762|MUP6_MOUSE ----MKMLLLLCGLTLVLCVHAEEASSTGRNFNVEKINGEWHTIILASDKREKIEDNGNF      56
                        **:* :*:***:*.* :*: * :* :*:***:* :

EMBOSS_001          RLFLEQIHVLEKSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYD      98
sp|Q5FW60|MUP20_MOUSE RVFVEYIHVLENSLALKFHIIINEECSEIFLVADKTEKAGEYSVTYDGSNTFTILKTDYD      117
tr|A2AV72|A2AV72_MOUSE RLFVEHIHVLENSLGFKFHTVIDEECSEIFLVADKTEKAGEYSVTYDGFKKFTVLKTDYD      115
sp|P11591|MUP5_MOUSE RVFVEHIDVLENSLAFKFHTVIDEECTEIIYLADKTEKAGEYSVTYDGFNTFTILKTDYD      116
sp|P11590|MUP4_MOUSE RVFVEHIHVLENSLAFKFHTVIDGECSEIFLVADKTEKAGEYSVMYDGFNTFTILKTDYD      114
tr|Q3KQQ2|Q3KQQ2_MOUSE RAFVENITVLENSLVFKFHLIVNEECTEMTAIGEQTAKAGIYYMNYDGFNTFSILKTDYD      120
sp|P04939|MUP3_MOUSE RAFVENITVLENSLVFKFHLIVNEECTEMTAIGEQTAKAGIYYMNYDGFNTFSILKTDYD      120
tr|Q80YX8|Q80YX8_MOUSE RAFVENITVLENSLVFKFHLIVNEECTEMTLIGEETAKAGIYYLNYDGFNTFTILKTDYD      117
sp|P02762|MUP6_MOUSE RLFLEQIHVLENSLVLKFHTVRDEECSELSMVADKTEKAGEYSVTYDGFNTFTIPKTDYD      116
                        * :* * :***:* :*** : : * :* : : : :***** * : *** :* : : *****

EMBOSS_001          NFLMAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCL      158
sp|Q5FW60|MUP20_MOUSE NYIMIHLINKKDGETFQLMELYGREPDLSSDIKEKFAQLSEEHGIVRENIIDLTNANRCL      177
tr|A2AV72|A2AV72_MOUSE NYIMFHLINEMNGETFQLMSLYGREPDLNSDIKEKFVKLCEEHGIIRENIIDFTKTNRCL      175
sp|P11591|MUP5_MOUSE NYIMFHLINKKDEENFQLMELFGREPDLSSDIKEKFAKLCEEHGIVRENIIDLSNANRCL      176
sp|P11590|MUP4_MOUSE NYIMFHLINEKDGETFQLMELYGRKADLNSDIKEKFVKLCEEHGIIRENIIDLTNTNRCL      174
tr|Q3KQQ2|Q3KQQ2_MOUSE NYIMIHLINKKDGETFQLMELYGREPDLSDIKEKFAKLCEEHGIIRENIIDLTVNVRCL      180
sp|P04939|MUP3_MOUSE NYIMIHLINKKDGETFQLMELYGREPDLSDIKEKFAKLCEEHGIIRENIIDLTVNVRCL      180
tr|Q80YX8|Q80YX8_MOUSE NYIMIYLINEKDGETFQLMELYGREPYLSLDIKEKFAKLCEEHGIIRENIIDLTVNVRCL      177
sp|P02762|MUP6_MOUSE NFLMAHLINEKDGETFQLMGLYGREPDLSSDIKERFAQLCEEHGILRENIIDLSNANRCL      176
                        * :* :***: : : :***** * :* : * :***** :* :* :***** : : :*****

EMBOSS_001          QARE*          162
sp|Q5FW60|MUP20_MOUSE EARE -          181
tr|A2AV72|A2AV72_MOUSE QARE -          179
sp|P11591|MUP5_MOUSE QARE -          180
sp|P11590|MUP4_MOUSE KARE -          178
tr|Q3KQQ2|Q3KQQ2_MOUSE EARE -          184
sp|P04939|MUP3_MOUSE EARE -          184
tr|Q80YX8|Q80YX8_MOUSE EARE -          181
sp|P02762|MUP6_MOUSE QARE -          180
                        :***

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Figure S2. Amino acid sequence alignment of Mus m 1.0102 allergen (a *central* Major Urinary Protein) and *peripheral* Major Urinary Proteins. Align tool, running the Clustal Omega algorithm to find sections of identity/similarity, was used for multiple sequence alignment. Consensus symbols: asterisk indicates a position which has a single, fully conserved residue; colon indicates a position which has at least one substitution between amino acids with similar properties (roughly equivalent to scoring > 0.5 in the Gonnet PAM 250 matrix [27]); period indicates a position which has almost one substitution between amino acids of weakly similar properties (roughly equivalent to scoring ≤ 0.5 and >0 in the Gonnet PAM 250 matrix); absence of a symbol corresponds to non-conservative substitutions. Each protein is represented by its accession number in UniProtKB database, while the query protein rMus m 1.0102 is indicated as EMBOSS_001 (dashed line at the beginning of the sequence indicates omission of the secretion signal).

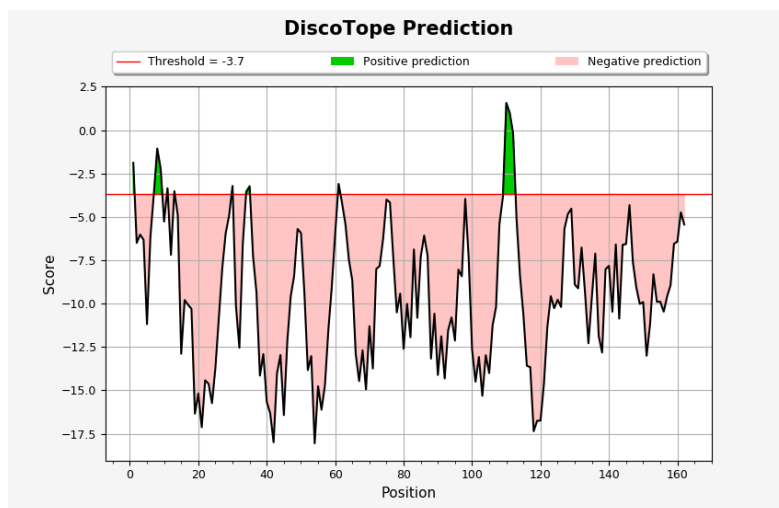


Figure S3. Graphical output of the B-cell epitope prediction by Discotope 2.0.

Table S1. List of the residues predicted by Discotope 2.0 as parts of B-cell epitopes

| Residue | Contact No. | Propensity score | Discotope score |
|-------------|-------------|------------------|-----------------|
| E1* | 8 | -1.089 | -1.884 |
| G7 | 4 | -3.642 | -3.684 |
| R8 | 8 | -0.156 | -1.058 |
| N9 | 8 | -1.465 | -2.217 |
| N11 | 10 | -2.486 | -3.351 |
| E13 | 1 | -3.848 | -3.521 |
| E30 | 4 | -3.115 | -3.217 |
| D34 | 1 | -3.884 | -3.553 |
| N35 | 3 | -3.262 | -3.232 |
| D61 | 0 | -3.502 | -3.1 |
| D110 | 1 | 1.903 | 1.569 |
| G111 | 0 | 1.136 | 1.006 |
| E112 | 4 | 0.293 | -0.201 |

* The color code corresponds to epitope highlighting in Figure 1C and 1D.

Table S2. List of residue scores obtained by SEPPA 3.0 server

| chainID | resSeq | resName | score* | location |
|----------|----------|------------|--------------|------------------|
| A | 1 | GLU | 0.065 | surface |
| A | 2 | GLU | 0.056 | surface |
| A | 3 | ALA | 0.056 | surface |
| A | 4 | SER | 0.069 | surface |
| A | 5 | SER | 0.057 | surface |
| A | 6 | THR | 0.064 | surface |
| A | 7 | GLY | 0.078 | surface |
| A | 8 | ARG | 0.094 | surface § |
| A | 9 | ASN | 0.069 | surface |
| A | 10 | PHE | 0.070 | surface |
| A | 11 | ASN | 0.072 | surface |
| A | 12 | VAL | 0.055 | surface |
| A | 13 | GLU | 0.052 | surface |
| A | 14 | LYS | 0.062 | surface |
| A | 15 | ILE | 0.000 | core |
| A | 16 | ASN | 0.048 | surface |
| A | 17 | GLY | 0.046 | surface |
| A | 18 | GLU | 0.057 | surface |
| A | 19 | TRP | 0.000 | core |
| A | 20 | HIS | 0.071 | surface |
| A | 21 | THR | 0.000 | core |
| A | 22 | ILE | 0.000 | core |
| A | 23 | ILE | 0.051 | surface |
| A | 24 | LEU | 0.041 | surface |
| A | 25 | ALA | 0.000 | core |
| A | 26 | SER | 0.000 | core |
| A | 27 | ASP | 0.049 | surface |
| A | 28 | LYS | 0.071 | surface |
| A | 29 | ARG | 0.059 | surface |
| A | 30 | GLU | 0.070 | surface |
| A | 31 | LYS | 0.067 | surface |
| A | 32 | ILE | 0.000 | core |
| A | 33 | GLU | 0.068 | surface |
| A | 34 | ASP | 0.077 | surface |
| A | 35 | ASN | 0.079 | surface |
| A | 36 | GLY | 0.062 | surface |
| A | 37 | ASN | 0.054 | surface |
| A | 38 | PHE | 0.048 | surface |
| A | 39 | ARG | 0.066 | surface |
| A | 40 | LEU | 0.042 | surface |
| A | 41 | PHE | 0.061 | surface |
| A | 42 | LEU | 0.039 | surface |
| A | 43 | GLU | 0.041 | surface |
| A | 44 | GLN | 0.037 | surface |
| A | 45 | ILE | 0.038 | surface |
| A | 46 | HIS | 0.041 | surface |
| A | 47 | VAL | 0.048 | surface |
| A | 48 | LEU | 0.056 | surface |
| A | 49 | GLU | 0.060 | surface |
| A | 50 | LYS | 0.059 | surface |
| A | 51 | SER | 0.057 | surface |
| A | 52 | LEU | 0.000 | core |
| A | 53 | VAL | 0.040 | surface |
| A | 54 | LEU | 0.033 | surface |
| A | 55 | LYS | 0.038 | surface |
| A | 56 | PHE | 0.040 | surface |
| A | 57 | HIS | 0.064 | surface |
| A | 58 | THR | 0.086 | surface |

| | | | | |
|---|-----|-----|-------|------------|
| A | 59 | VAL | 0.129 | surface |
| A | 60 | ARG | 0.141 | surface |
| A | 61 | ASP | 0.197 | surface \$ |
| A | 62 | GLU | 0.171 | surface |
| A | 63 | GLU | 0.153 | surface |
| A | 64 | CYS | 0.130 | surface |
| A | 65 | SER | 0.099 | surface |
| A | 66 | GLU | 0.055 | surface |
| A | 67 | LEU | 0.043 | surface |
| A | 68 | SER | 0.035 | surface |
| A | 69 | MET | 0.035 | surface |
| A | 70 | VAL | 0.046 | surface |
| A | 71 | ALA | 0.000 | core |
| A | 72 | ASP | 0.055 | surface |
| A | 73 | LYS | 0.061 | surface |
| A | 74 | THR | 0.057 | surface |
| A | 75 | GLU | 0.060 | surface |
| A | 76 | LYS | 0.068 | surface |
| A | 77 | ALA | 0.064 | surface |
| A | 78 | GLY | 0.000 | core |
| A | 79 | GLU | 0.053 | surface |
| A | 80 | TYR | 0.000 | core |
| A | 81 | SER | 0.049 | surface |
| A | 82 | VAL | 0.044 | surface |
| A | 83 | THR | 0.050 | surface |
| A | 84 | TYR | 0.047 | surface |
| A | 85 | ASP | 0.069 | surface |
| A | 86 | GLY | 0.120 | surface |
| A | 87 | PHE | 0.065 | surface |
| A | 88 | ASN | 0.000 | core |
| A | 89 | THR | 0.046 | surface |
| A | 90 | PHE | 0.034 | surface |
| A | 91 | THR | 0.036 | surface |
| A | 92 | ILE | 0.038 | surface |
| A | 93 | PRO | 0.040 | surface |
| A | 94 | LYS | 0.039 | surface |
| A | 95 | THR | 0.000 | core |
| A | 96 | ASP | 0.043 | surface |
| A | 97 | TYR | 0.048 | surface |
| A | 98 | ASP | 0.047 | surface |
| A | 99 | ASN | 0.049 | surface |
| A | 100 | PHE | 0.048 | surface |
| A | 101 | LEU | 0.036 | surface |
| A | 102 | MET | 0.042 | surface |
| A | 103 | ALA | 0.031 | surface |
| A | 104 | HIS | 0.033 | surface |
| A | 105 | LEU | 0.034 | surface |
| A | 106 | ILE | 0.053 | surface |
| A | 107 | ASN | 0.000 | core |
| A | 108 | GLU | 0.144 | surface |
| A | 109 | LYS | 0.142 | surface |
| A | 110 | ASP | 0.169 | surface \$ |
| A | 111 | GLY | 0.220 | surface \$ |
| A | 112 | GLU | 0.196 | surface \$ |
| A | 113 | THR | 0.128 | surface |
| A | 114 | PHE | 0.081 | surface |
| A | 115 | GLN | 0.052 | surface |
| A | 116 | LEU | 0.039 | surface |
| A | 117 | MET | 0.000 | core |
| A | 118 | GLY | 0.000 | core |
| A | 119 | LEU | 0.000 | core |
| A | 120 | TYR | 0.039 | surface |
| A | 121 | GLY | 0.000 | core |

| | | | | |
|----------|------------|------------|--------------|----------------|
| A | 122 | ARG | 0.054 | surface |
| A | 123 | GLU | 0.073 | surface |
| A | 124 | PRO | 0.093 | surface |
| A | 125 | ASP | 0.104 | surface |
| A | 126 | LEU | 0.069 | surface |
| A | 127 | SER | 0.080 | surface |
| A | 128 | SER | 0.075 | surface |
| A | 129 | ASP | 0.075 | surface |
| A | 130 | ILE | 0.063 | surface |
| A | 131 | LYS | 0.072 | surface |
| A | 132 | GLU | 0.067 | surface |
| A | 133 | ARG | 0.060 | surface |
| A | 134 | PHE | 0.000 | core |
| A | 135 | ALA | 0.058 | surface |
| A | 136 | GLN | 0.056 | surface |
| A | 137 | LEU | 0.047 | surface |
| A | 138 | CYS | 0.000 | core |
| A | 139 | GLU | 0.046 | surface |
| A | 140 | GLU | 0.046 | surface |
| A | 141 | HIS | 0.043 | surface |
| A | 142 | GLY | 0.048 | surface |
| A | 143 | ILE | 0.046 | surface |
| A | 144 | LEU | 0.050 | surface |
| A | 145 | ARG | 0.048 | surface |
| A | 146 | GLU | 0.050 | surface |
| A | 147 | ASN | 0.050 | surface |
| A | 148 | ILE | 0.046 | surface |
| A | 149 | ILE | 0.056 | surface |
| A | 150 | ASP | 0.069 | surface |
| A | 151 | LEU | 0.065 | surface |
| A | 152 | SER | 0.093 | surface |
| A | 153 | ASN | 0.107 | surface |
| A | 154 | ALA | 0.074 | surface |
| A | 155 | ASN | 0.088 | surface |
| A | 156 | ARG | 0.108 | surface |
| A | 157 | CYS | 0.112 | surface |
| A | 158 | LEU | 0.114 | surface |
| A | 159 | GLN | 0.138 | surface |
| A | 160 | ALA | 0.145 | surface |
| A | 161 | ARG | 0.136 | surface |
| A | 162 | GLU | 0.124 | surface |

*Residues with a score higher than the threshold (0.089) are predicted as epitope residues (*in bold*).

[§]Residues in blue, red, and green were predicted also by Discotope 2.0 as parts of B-cell epitopes (see Figure 1, epitopes I, II, III).

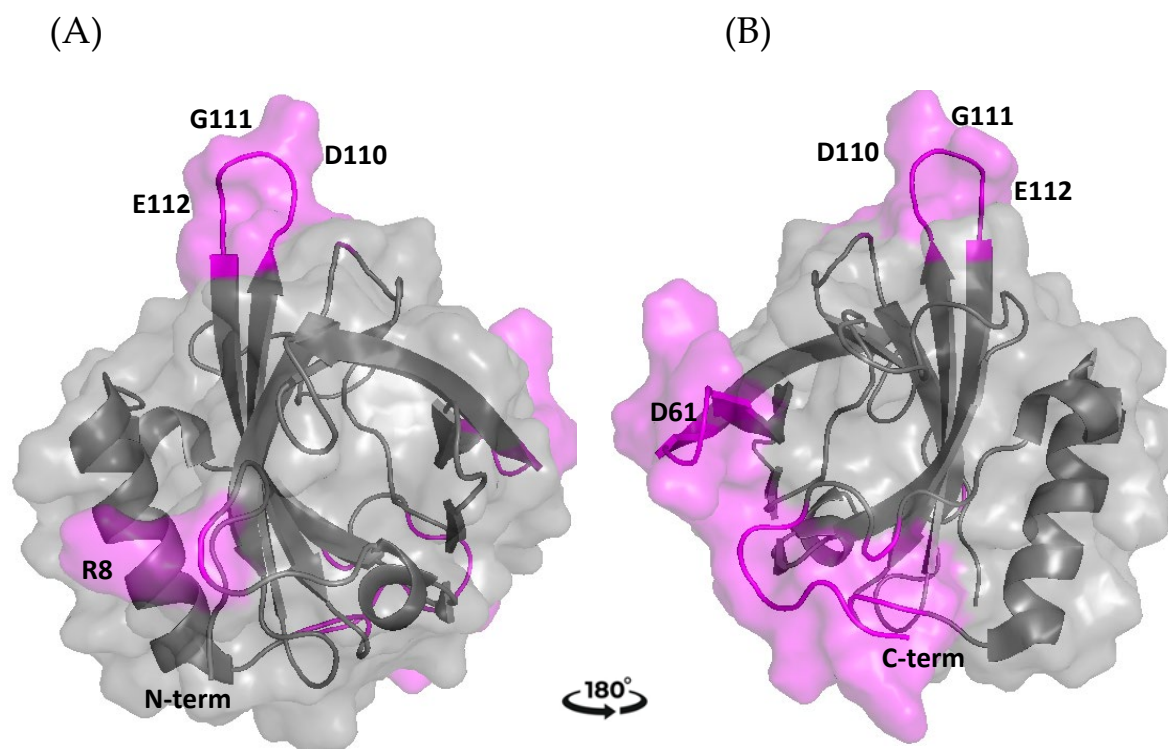
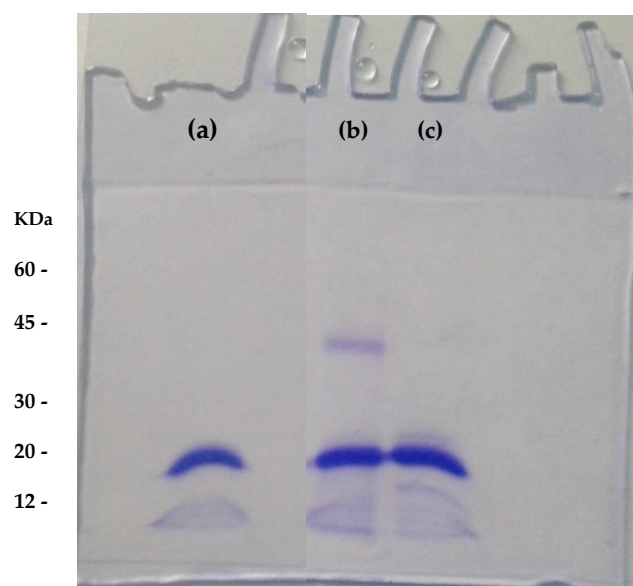
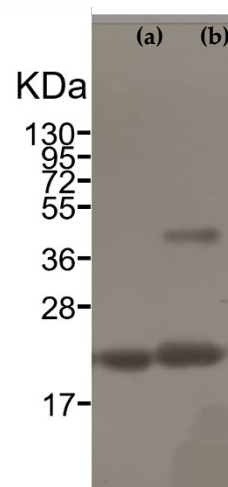


Figure S4. Conformational epitopes predicted by SEPPA 3.0. (A,B) The magenta regions of surface and cartoon representations of rMus m 1.0102 solution structure (PDB code 1df3, model 1) correspond to predicted B-cell epitopes. Labeled epitope residues (R8, D61, D110, G111, E112) were predicted also by Discotope 2.0 as parts of B-cell epitopes (Figure 1C,D). (A) bottom view of the central cavity, (B) top view of the cavity, generated by a 180° turn.

(A)



(B)



(C)

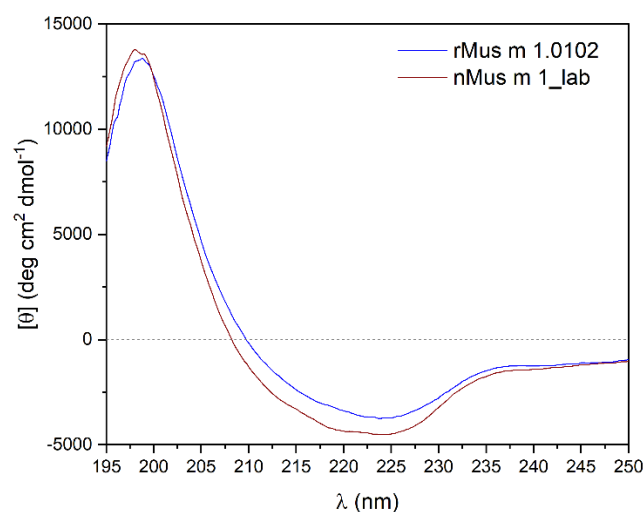


Figure S5. Protein characterization. (A) SDS-PAGE and (B) western blot analyses of rMus m 1.0102 (lanes a), laboratory (lanes b), and commercial (Indoor Biotechnologies) (lane c) preparations of urinary nMus m 1. Apparently, the band at 40 kDa of the laboratory preparation of nMus m 1 might be ascribed to a dimer formation, possibly due to a cysteine reoxidation reaction. The low molecular weight bands appreciable at <20 kDa of nMus m 1 commercial preparation sample might be due to a partial degradation process. (C) CD spectra for rMus m 1.0102 and nMus m 1 laboratory preparation samples, acquired in the far-UV region.

Table S3. Correlation analysis of indirect ELISA data

| Variables | Spearman corr. Coefficient | p-Value |
|--|----------------------------|----------|
| <i>- ELISA data:</i> | | |
| rMus m 1.0102/ ^a nMus m 1 | 0.51496 | 0.02875* |
| rMus m 1.0102/ ^b nMus m 1 | 0.43627 | 0.07999 |
| ^a nMus m 1/ ^b nMus m 1 | 0.64461 | 0.00521* |
| <i>- ELISA data vs. ImmunoCAP ISAC data:</i> | | |
| rMus m 1.0102/Mus m 1-specific IgE | 0.55005 | 0.01803* |

* Significant *p*-value at the 0.05 level
^a nMus m 1 laboratory preparation; ^b nMus m 1 commercial preparation.

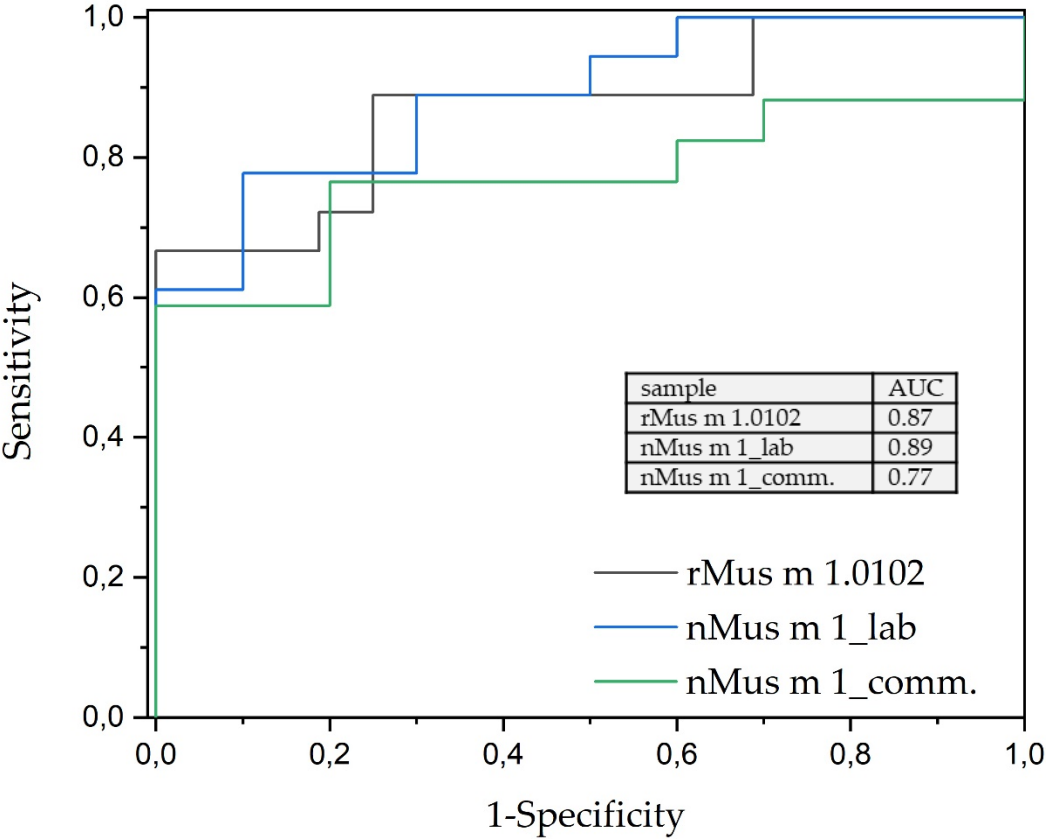


Figure S6. ROC curves of ELISA data based on rMus m 1.0102 and urinary nMus m 1 allergens (laboratory and commercial preparations). Data were obtained with control and allergic sera.