Title: Tropomyosin in mugwort cross-reacts to house dust mite, eliciting non-Th2 response in allergic rhinitis patients sensitized to house dust mite

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Conflict of interest: The authors declare that they have no conflict of interest.

Manuscript word count: 4393, **Table:** 2, **Figure:** 3

Short title: Cross-reactivity between mugwort and HDM tropomyosin

24 ABSTRACT

25 **Background:** Mugwort and house dust mite (HDM) are two of the most common
26 inhalant allergens in Asia; however, whether or not mugwort affects polysensitized
27 HDM⁺ allergic rhinitis (AR) patients has not been elucidated.

28 **Methods:** Overall, 15884 AR outpatients were assessed for clinical status. Amino acid
29 sequences of mugwort were determined by mass spectrometry. Afterward, cross-
30 reactivity between mugwort tropomyosin and Dermatophagoides pteronyssinus 10
31 (Der p10) was analysed by ELISA inhibition and basophils activation experiments. To
32 compare immunologic responses eliciting by two different tropomyosins, peripheral
33 blood mononuclear cells (PBMCs) of HDM-monosensitized patients were stimulated
34 by mugwort, HDM, Der p10 and synthetic peptides representing mugwort
35 tropomyosin respectively.

36 **Results:** Polysensitized HDM⁺AR patients were mainly sensitized to cat and
37 mugwort, and the positive rate of monosensitized HDM⁺AR out-clinic patients was
38 increased during the mugwort pollen season. Mugwort tropomyosin protein had
39 similar structural domains to HDM tropomyosin, Der p10. ELISA inhibition
40 experiment showed synthetic mugwort tropomyosin peptide inhibited IgE binding to
41 Der p10; mugwort tropomyosin peptide activated basophils which were primed by
42 HDM-specific IgE. Unlike HDM and Derp 10, mugwort and mugwort tropomyosin
43 mainly induced IFN- γ and IL-17, release in PBMCs of monosensitized HDM⁺AR
44 patients, but not IL-5.

45 **Conclusions:** Pan-allergen tropomyosin is a major protein accounting for the cross-
46 reactivity between mugwort and HDM, which reminds HDM⁺ patients to reduce

47 mugwort exposure in mugwort pollen season in virtue of the tropomyosin induced

48 mild inflammation.

49 **Keywords:** cross-reactivity, house dust mite, mugwort

50 INTRODUCTION

51 Allergic rhinitis (AR) is an upper airway allergic inflammatory disease, which causes
 52 symptoms of sneeze, runny nose, nasal obstruction and itchy nose, and is mediated by
 53 type 2 helper (Th2) cells and immunoglobulin E (IgE)^{1,2}. Among the common
 54 triggering allergens, house dust mites (HDM), mould spores and animal dander
 55 mainly cause symptoms of perennial allergic rhinitis, whereas, a large variety of
 56 pollens from different geographical regions contributes to symptoms of seasonal
 57 allergic rhinitis³. Some AR patients are found to be polysensitized to more than one
 58 allergen⁴, and an increasing number of sensitizations strongly predisposes AR patients
 59 to allergic asthma^{5,6}. Thus, the treatment for polysensitized AR patients has a close
 60 relationship with asthma management⁷.

61 Allergen specific immunotherapy (AIT) is an effective therapeutic method for
 62 monosensitized AR patients⁷. However, management approaches to polysensitized
 63 AR patients by AIT are not standardized yet. There are intercontinental differences in
 64 allergen products available for AIT in polysensitized patients⁸. Desensitization to the
 65 most clinically relevant allergen is often used to treat polysensitized patients in
 66 Europe and in China, while mixtures of extracts are recommended in the United
 67 States^{9,10}. Differences in therapeutic effects of single allergen-specific immunotherapy
 68 have been shown: more effective in reducing the symptoms are observed in those of
 69 monosensitized patients than that of polysensitized patients treated with the same
 70 dose^{11,12}; no obvious change in HDM-specific IgE production and a lower
 71 concentration of HDM-specific IgG4 is noticed in polysensitized patients compared
 72 with those of monosensitized patients after AIT^{12,13}. Polysensitization is mainly
 73 caused by cross-reactivity among closely related allergens, or allergens from other
 74 sources. The identification of primary causal allergen(s) and sensitization to cross-

75 reacting allergens helps us find efficient ways to treat polysensitized AR patients in
76 the near future.

77 HDM and mugwort have been regarded as the two most common and clinically
78 relevant sensitizing allergens in AR patients in Asia ¹⁴. HDM cross-reacts with
79 allergens from other invertebrates, including other species of mites, insects, mollusks,
80 and crustaceans¹⁵. It is not clear whether or not there is cross-reactivity between HDM
81 and mugwort; consequently, whether or not mugwort affects polysensitized HDM⁺AR
82 patients. Thus, it would clearly be of interest to explore whether HDM and mugwort
83 share similar structural features that elicit a common immunologic response in
84 polysensitized and monosensitized patients. In view of this, the present study has
85 specifically investigated cross-reactivity between HDM and mugwort in HDM⁺AR
86 patients.

87 MATERIAL AND METHODS

88 Study design and subjects

89 Subjects with AR based on criteria of the Allergic Rhinitis and its Impact on Asthma
 90 (ARIA) consensus statement¹⁶ were recruited consecutively from the allergy-
 91 rhinology outpatient clinic of Beijing Tongren Hospital. On recruitment, each subject
 92 completed a questionnaire to record demographic data, nasal symptom severity, and
 93 history of asthma; and blood samples were collected from each subject for analysis of
 94 serum specific IgE antibodies. Peripheral mononuclear cells (PBMCs) were also
 95 prepared from blood samples of some HDM⁺AR patients and healthy controls for this
 96 study. None of the subjects had received any allergen-specific immunotherapy or
 97 monoclonal antibody treatment. The study was approved by the Medical Ethics
 98 Committee of Beijing Tongren Hospital, and all patients provided written informed
 99 consent before entry into the study and collection of any samples.

100 Serum antigen-specific IgE measurements

101 The presence of IgE antibodies in blood was determined using a EUROLINE Atopy
 102 Screen (DP 3713 E; Lubeck Germany), which comprised two sets of allergens; one
 103 with a mix of aeroallergens [including tree mix (willow, poplar, elm), common
 104 ragweed, mugwort, house dust mite mix (*Dermatophagoides pteronyssinus* (Der p),
 105 *Dermatophagoides farina* (Der f), house dust, cat, dog, cockroach German, mould mix
 106 (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria*
 107 *alternata*) and hops], and one with a mix of food allergens [including egg white,
 108 cow's milk, peanut, soybean, beef, mutton, sea fish mix (codfish, lobster, scallop),
 109 shrimp, and crab]. Furthermore, concentrations of Der f2 specific IgE, Der p1 specific
 110 IgE, and total IgE were also measured using the ImmunoCAP system

111 (Immunodiagnosics; Thermo Fisher Scientific, Uppsala, Sweden). Allergen-specific
112 IgE >0.35 kU/L was considered as positive.

113 **Mass Spectrometry**

114 Prior to analysis, 100 mg samples of mugwort (*Artemisia annua* (A. annua) and
115 *Artemisia sieversian* (A. sieversian)) were separately prepared as peptide solutions by
116 denaturing and treatment with protease trypsin according to the method described by
117 León and colleagues¹⁷; and then analysed in a Triple-TOF 6600 mass spectrometer
118 (Sciex, United States) fitted with a Nanospray III source (Sciex). The ion spray
119 voltage was 2300 V, declustering potential 80 V, curtain gas 35 psi, nebulizer gas
120 5psi, and interface heater temperature 150 °C. The peptides were introduced into the
121 mass spectrometer via Nona 415 liquid chromatography column (Sciex) eluted with
122 water/acetonitrile/formic acid (buffer B: 2/98/0.1%). In this regard, samples (4 µL)
123 were injected onto a C18 desalted column (3 µm, 120Å, 350 µm×0.5 mm), and
124 separated onto a C18 analysis column (3 µm, 120Å, 75 µm×150 mm) with gradients
125 ranging from 5 to 16% buffer B in the first 25 min, from 16 to 26% buffer B in the
126 next 20 min, from 26 to 40% buffer B in the following 3 min, from 40 to 80% buffer
127 B in the next 5 min, and finally from 80 to 5% buffer B in the final 7 mins; at a flow
128 rate of 0.6 µL/min. The peptides present in the samples, were matched to the UniProt
129 *Artemisia annua* databases, and identified for the corresponding proteins. A
130 standardised preparation of common repeat peptide sequences of tropomyosin protein
131 from both A. annua and A. sieversian (SynPeptide, Shanghai, China) was selected for
132 further functional analysis as follows: VGSPDESYEDFTNSLPSNECR.

133 **Comparative modelling of three-dimensional (3D) protein structures**

134 Homology modelling for tropomyosin of mugwort (*A. annua* and *A. sieversian*) was
135 established on the SWISS-MODEL server. The modelling template was selected from
136 RCSB PDB Data Bank (PDB ID: 1f7s and 1xwv) and molecular dynamics
137 simulations were carried out for mugwort using AMBER ff14SB force field. The
138 structures were explicitly solvated with a water box from the outermost atoms of the
139 molecules. Periodic boundary conditions were used and the net negative charge
140 neutralized with Na⁺ counterion. The best model was selected for the analysis of the
141 similarity between tropomyosin of mugwort and Der p10. The structure of Der p10
142 was established on the SWISS-MODEL server.

143 **HDM-specific IgE blockage by synthesised mugwort tropomyosin peptides**

144 Serum samples of 15 HDM⁺AR patients each with a high or low level of HDM-
145 specific IgE were used to assess the IgE blocking potential of synthetic peptides of
146 mugwort tropomyosin. Briefly, 200 µL of serum from HDM⁺AR patients were
147 incubated with or without mugwort tropomyosin peptides (1000 ng/mL for each) for 1
148 h at room temperature, and at the end of incubation the serum samples were analysed
149 for the concentrations of Der p1-specific IgE and Der f2-specific IgE using the
150 ImmunoCAP system.

151 **Basophil activation test**

152 PBMCs isolated from non-allergic donors, 5×10⁵ cells were stripped in 2ml ice cold
153 lactic acid buffer (0.13M KCl, 0.05M NaCl, 0.01M lactic acid, pH=3.9) for 30s as
154 described before¹⁸. After washing 3 times by PBS, cells were pre-incubated with sera
155 from HDM-allergic individuals for 1 h at 37°C. And then, cells were stimulated by
156 different concentrations of mugwort tropomyosin or Derp 10 (50, 500 ng/mL) in
157 hepes buffer containing IL-3 (R&D, Minneapolis, Minnesota, USA). In the

meanwhile, cells exposed to FLMP (Sigma, St. Louis, USA)) were taken as a positive control. The reaction was stopped by EDTA buffer (20 mM). In the end, PBMCs were stained with basophil surface markers: CD123BV650, CCR3-APC-fire750 and CD63-PE (Biolegend, San Diego, CA, USA), and the percentages of CD63⁺CD123⁺CCR3⁺ cells were analysed by Flowjo software.

ELISA inhibition experiment

Plates were pre-coated with Der p10 obtained from CUSABIO company (Wuhan, China) overnight at 4°C. Non-specific binding in the well was reduced by incubation with PBS supplemented with 1% BSA and 0.05% tween 20 for 6 h at room temperature. Inhibition was performed by adding sera from HDM monosensitized patients with synthetic mugwort tropomyosin peptides (50, 500 ng/mL), and sera without peptides were taken as non-inhibition conditions. Anti-human IgE (2 ug/mL, NOVUS, USA) were added, followed by streptavidin-HRP conjugated secondary antibodies (diluted 1:2,000; EasyBio, Beijing, China). Absorbance at 405 nm was determined using an ELISA reader (BioTek, Vermont, USA). All experiments were performed in duplicate. Percent inhibition was calculated using the following equation: percent inhibition = 100- [(OD of serum with tropomyosin peptide /OD of serum without peptide) × 100].

Stimulation of PBMCs *ex vivo*

PBMCs were isolated from the blood of 6 healthy and 12 AR donors using Ficoll-Hypaque density gradient centrifugation according to the standard protocol (LymphoprepTM, Nycomed Pharma, Oslo, Norway). Cells were plated at a density of 1x10⁶ cells/well in a 24-well plate in 0.5 mL RPMI 1640 (Gibco, USA) culture medium alone, or with HDM (Der p1 extract; 0.2, 1, 5 µg/mL; GREER Laboratories,

182 Lenoir, NC, USA), mugwort (1, 10, 100, 1000 ng/mL; locally prepared in Beijing
183 Tongren Hospital), or mugwort tropomyosin peptides in the culture medium, and then
184 incubated at 37 °C in 5% CO₂ for 48 h. At the end of incubation, the cell suspensions
185 were collected and the supernatants were assessed for IL-5, IL-17, and IFN- γ using
186 Luminex xMAP suspension array technology in a Bio-Plex 200 system (Bio-Rad, MI).
187 All cytokine kits were purchased from R&D Company and the cytokines were
188 expressed as pg/mL.

189 **Statistical analysis**

190 Statistical analysis was performed using the SPSS version 22.0 software package
191 (IBMCorp, Armonk, NY, USA). Categorical variables were described using
192 frequencies and/or percentages and continuous variables were presented as means $\pm$
193 standard deviation (SD). Multiple logistic regression was used to analyse the possible
194 risk factors for polysensitized HDM⁺AR patients. The influence of polysensitization
195 on asthma development was assessed by the Chi-squared test. The prevalence of
196 different allergens in HDM⁺AR patients was estimated using Fisher's exact test and
197 logistic regression. The Wilcoxon test was used for paired between-group
198 comparisons of the effect of specific antigen stimulation on the release of cytokines
199 from PBMCs, and the effect of mugwort peptides on blocking Der p1 and Der f2
200 sIgEs. *P* values of less than 0.05 were regarded as statistically significant.

201 **RESULTS**

202 **Mugwort may affect the prevalence of HDM⁺AR patients**

203 A total of 497 HDM⁺AR patients were recruited into the study. Overall, 64.6% of the
 204 HDM⁺AR patients were monosensitized, and 35.4% polysensitized (**Table 1**).
 205 Comorbid asthma was more prevalent in 19.32% of all polysensitized AR patients
 206 compared to in 9.03% of all monosensitized AR patients ($p = 0.001$). Type of
 207 sensitizing allergens was further analysed in polysensitized HDM⁺AR patients in
 208 parallel with the HDM-specific IgE level. Regardless of the level of HDM-specific
 209 IgE detected, sensitization was greatest to inhalant allergens in the polysensitized
 210 HDM⁺AR patients (**Figure 1A**). The five most prevalent inhalant allergens in the
 211 polysensitized HDM⁺AR patients were cat (27.8%, 95% CI:21.2%-34.5%), mugwort
 212 (26.1%, 95% CI:19.6%-32.7%), house dust (21.6%, 95% CI:15.5%-27.7%),
 213 cockroach (20.5%, 95% CI:14.4%-26.5%) and hops (10.8%, 95% CI: 6.2%-15.4%)
 214 (**Figure 1B**). The number of monosensitized HDM⁺AR patients was increased from
 215 July to August, and the number of polysensitized HDM⁺AR patients from July to
 216 September (**Figure 1C**); which appeared to follow the trend of the mugwort pollen
 217 season seen from July to early September in 2018¹⁹.

218 **Tropomyosin was involved in mugwort-HDM cross-reactivity due to the similar** 219 **structure**

220 Alignment of peptide amino acid sequences of *A. annua* and *A. sieversian* allergens in
 221 UniProt *Artemisia annua* databases indicated the presence of cross-reactivity protein
 222 tropomyosin (**Table 2**). Similarly, the presence of cross-reactivity proteins profilin
 223 and lipid transfer protein were also found in both *A. annua* and *A. sieversian* (data not
 224 shown). However, alignment analysis of the amino acid sequences demonstrated for

the tropomyosin proteins in mugwort with the amino acid sequences for Der p10 (a member of the tropomyosin group protein in HDM), showed that there was no homology. Comparative 3D-modeling of the tropomyosin protein structures of mugwort vs Der p10, optimized using AMBER14 molecular dynamics software, demonstrated that the tropomyosin proteins in mugwort contained α -helices and β -sheets, which were comparable to Der p10 (**Figure 2A**).

Pre-incubation serum samples of monosensitized HDM⁺AR patients with mugwort tropomyosin peptide significantly decreased the concentrations of HDM specific-sIgE in the serum (**Figure 2B**). Furthermore, mugwort tropomyosin peptide (50 ng/ml) inhibited IgE binding to Der p10 ranging from 2.4% to 32.1% (**Figure 2C**). As shown in **Figure 2D**, the activation of basophils, which pre-sensitized by HDM specific IgE, occurred with exposure to Der p10 and synthetic tropomyosin mugwort peptide in 2 out of 8 non-allergic patients, suggesting the similar structure domains of tropomyosin in mugwort and HDM are functional.

A. annua and synthetic tropomyosin of mugwort induced non-Th2 response in PBMCs of monosensitized HDM⁺AR patients

Compared to medium controls, HDM stimulated PBMCs isolated from monosensitized HDM⁺AR patients (n = 6) to produce high levels of IL-5 (0.59-61.56 pg/mL) and IL-17 (0.91-5.27 pg/mL), but not IFN- γ (**Figure 3A**). In contrast, HDM induced PBMCs isolated from healthy controls (n = 6) to release IL-17 (0.71-1.32 pg/mL) and IFN- γ (0.71-1.79 pg/mL), but not IL-5 (**Figure 3A**). Interestingly, *A. annua* stimulated PBMCs from monosensitized HDM⁺AR patients (n = 8) to produce IL-17 (1.36-2.24 pg/mL) and IFN- γ (3.7-4.79 pg/mL), but not IL-5 (**Figure 3B**); while such stimulation only induced the production of IFN- γ (1.79-47.45 pg/mL), but

249 not IL-5 or IL-17 by PBMC of healthy controls (n = 5) (**Figure 3B**). Generally, the
 250 frequency of *A. annua*-induced release of IL-17 and IFN- γ was 25% and 12.5%,
 251 respectively, from PBMCs of monosensitized HDM⁺AR patients; and the frequency
 252 of HDM-induced release of IL-5 and IL-17 was 66.7% and 83.3%, respectively, from
 253 PBMCs of monosensitized HDM⁺AR patients (**Figure 3C**), suggesting that HDM is
 254 more effective than *A. annua* in inducing inflammation in HDM⁺AR patients.

255 To confirm that tropomyosin may be responsible for *A. annua* extract-induced non-
 256 Th2 response in monosensitized HDM⁺AR patients, PBMCs from monosensitized
 257 HDM⁺AR patients were incubated with tropomyosin mugwort peptides and Der p10.
 258 Finally, mugwort peptide induced the synthesis and release of IFN- γ , with about 20%
 259 frequency and 60% of subjects released IL-17; whereas Der p10 induces the
 260 frequency of IL-5 and IL-17 were 20% and 60% respectively (**Figure 3D**).

261 DISCUSSION

262 We demonstrated for the first time that cross-reactive protein tropomyosin in mugwort
263 and HDM sharing similar structural domains is responsible for the cross-reactivity
264 between HDM and mugwort. However, unlike HDM and Derp 10, *A. annua* and
265 mugwort tropomyosin peptide induced Th1 and Th17 in PBMCs of HDM
266 monosensitized AR patients, but not Th2 response.

267 Tropomyosin, a pan-allergen, belongs to a family of phylogenetically conserved
268 proteins with multiple isoforms present in muscle and non-muscle cells of vertebrates
269 and invertebrates²⁰. It has been known that tropomyosin from HDM and cockroaches
270 share high sequence homology with that of shellfish, which unsurprisingly results in
271 cross-reactivity among HDM, cockroach and food allergens²¹⁻²³. Mugwort is the most
272 important outdoor seasonal allergen in Asia^{4,24}. Our data have shown that a large
273 number of polysensitized HDM⁺AR patients are sensitized to mugwort and the
274 number of monosensitized HDM⁺AR patients was increased from July to August.
275 Mite densities indeed vary with seasons and areas. Reportedly, three peaks for the
276 domestic mites density in Beijing appear in September to October, January and May
277 ²⁵. Thus, the increased number of monosensitized HDM⁺AR patients in July and
278 August did be affected by mugwort. Although amino acid sequences of mugwort (*A.*
279 *annua* and *A. sieversian*) tropomyosin proteins are different from that of HDM
280 tropomyosin Der p10, 3D-modelling results show that the α -helices and β -sheets in
281 mugwort tropomyosin are similar to Der p10. Considering that the sequence of the
282 same protein varies in different species and cross-reactivity is thought to occur when a
283 protein of similar sequence, structure or family binds to T and B cell receptors²⁶,

284 tropomyosin might be involved in mugwort-HDM cross-reactivity due to similar
285 structure of tropomyosin in allergens.

286 The present study has indicated that stimulation of PBMCs of monosensitized
287 HDM⁺AR patients with mugwort induced synthesis of IL-17 and IFN- γ , whereas
288 stimulation with HDM induced synthesis of IL-5 and IL-17. Our group has previously
289 demonstrated that single-nucleotide polymorphisms (SNPs) in IL-17A and IL-17F
290 gene regions are potentially associated with the development of AR and comorbid
291 asthma in Chinese subjects²⁷. Similarly, a study in Caucasian subjects has also
292 demonstrated that there is an association between serum IL-17 and the severity of
293 clinical symptoms in AR patients²⁸. As mentioned above, the role of Th17 in the
294 pathogenesis of AR cannot be excluded. Thus, the induction of IL-17 by mugwort
295 from PBMCs in monosensitized HDM⁺AR patients may be associated with clinical
296 symptoms of patients. In this study, the finding for Der p10-induced synthesis of IL-5
297 in monosensitized HDM⁺AR patients was in accordance with the findings of
298 stimulation by HDM. In the meanwhile, stimulation synthesized tropomyosin peptide
299 of mugwort could induce IL-17 and IFN- γ by PBMCs of HDM⁺AR subjects,
300 suggesting that tropomyosin is responsible for the cross-reactivity between HDM and
301 mugwort, eliciting non Th2 response.

302 In conclusion, we have for the first time demonstrated that mugwort tropomyosin
303 shares similar α -helices and β -sheets with Der p10, and therefore might play a role in
304 eliciting a non-Th2 response in polysensitized HDM⁺AR patients in comparison to
305 HDM. Since mugwort stimulation may be related to clinical symptoms of HDM
306 sensitized patients, education and allergen avoid are required for HDM⁺AR patients in
307 the autumn pollen season.

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310 **Acknowledgements:** This work was supported by grants from the Beijing Nova
 311 Program of Science and Technology (Z191100001119117); Yong top-notch talent
 312 (2018000021223ZK12); the Beijing Hospitals Authority Youth Programme
 313 (QML20180201); Beijing Scientific and Technological overall plan
 314 (Z171100000117002); the Beijing advanced innovation center for food nutrition and
 315 human health (Beijing Technology and Business University, 20181045); and the
 316 Program for the Changjiang Scholars and Innovative Research Team (IRT13082).

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394

Table 1. Demographic and clinical characteristics of house dust mite-positive allergic rhinitis patients investigated.

	Monosensitization (n=321) (64.4%)	Polysensitization (n=176) (35.4%)	P value	Odds ratio	95%CI
Gender			0.058	0.690	0.470-1.012
Male	158 (49.22)	101 (57.39)			
Female	163 (50.78)	75 (42.61)			
Age	29.60±11.55	28.77±12.09	0.449		
Family history of AR	69 (21.50)	54 (30.68)	0.056	1.522	0.989-2.342
Smoking and drink	222 (69.16)	99 (56.25)	0.003*	0.560	0.381-0.823
Co-morbid Allergic status					
Asthma	29 (9.03)	34 (19.32)	0.001*	2.486	1.448-4.267
Atopic Dermatitis	28 (8.72)	24 (13.64)	0.118	1.506	0.818-2.772
Allergic conjunctivitis	34 (10.59)	17 (9.66)	0.206	0.648	0.331-1.269
HDM specific IgE (kU/L)	25.44±26.66	29.89±29.92	0.112	1.005	0.999-1.012

*P< 0.05, HDM= house dust mite.

Table 2: Amino acid sequences of *Artemisia annua* and *Artemisia sieversian* tropomyosin fragments detected by mass spectrometry.

Mugwort			
Species	Names	Conf. %	Sequence
<i>Artemisia annua</i>	Actin-binding, cofilin/tropomyosin type	99	VGSPDESYEDFTNSLPSNECR
	Actin-binding, cofilin/tropomyosin type	99	IEEQQVIVEK
<i>Artemisia sieversian</i>	Actin-binding, cofilin/tropomyosin type	99	IEEQQVIVEK
	Actin-binding, cofilin/tropomyosin type	99	VGSPDESYEDFTNSLPSNECR

402

403 **FIGURE LEGENDS**

404 **Figure 1. A :** The distribution of allergen types, according to the concentrations of
 405 house dust mite (HDM) specific IgE in polysensitized HDM⁺ allergic rhinitis (AR)
 406 patients. Food allergens included crab, shrimp, soybean, sea fish mix 1, egg white,
 407 beef, cow's milk, peanut and mutton; while inhalant allergens included cat, mould
 408 mix 1, mugwort, hops, common ragweed, dog, cockroach, German, tree mix 2 and
 409 house dust. **B :** The prevalence of inhalant allergens in polysensitized HDM⁺AR
 410 patients (n=176). **C :** The positive rate of monosensitized and polysensitized
 411 HDM⁺AR patients in recruited outpatients from January 2018 to December 2018
 412 (n=15354).

413 **Figure 2. A:** Structural comparison of tropomyosin of *Artemisia annua* (*A. annua*)
 414 and *Dermatophagoides pteronyssinus* 10 (Der p10). The target sequence of homology
 415 modelling tropomyosin of *A. annua* was established on the SWISS-MODEL server
 416 eEF1A2 (Swiss-Prot Accession No: Q05639). The amino acid sequences of each
 417 protein were then loaded into the SWISS-MODEL server to predict a 3D model. **B:**
 418 Concentrations of house dust mite (HDM)-specific IgE in the serum of HDM⁺ allergic
 419 rhinitis (AR) patients (n = 15) incubated in the absence or presence of synthesized
 420 tropomyosin peptides of mugwort; **C:** Tropomyosin of *Artemisia annua* (*A. annua*)
 421 peptide inhibits IgE-binding to *Dermatophagoides pteronyssinus* 10 (Der p10)
 422 measuring by ELISA. Tropomyosin of *A. annua* exhibits inhibitory effect in 4 out of 8
 423 patients at the concentration of 50 ng/mL. **D:** The expression of basophils
 424 (CCR3⁺CD123⁺CD63⁺) with stimulations of Der p10 and tropomyosin of *A. annua*

425 peptide stimulations. Totally, HDM specific IgE pre-incubated basophils from 2 out
426 of 8 patients were activated by tropomyosin of *A. annua*.

427 **Figure 3.** Effect of specific antigen on release of IL-17, IL-5 and IFN- γ in peripheral
428 blood mononuclear cells (PBMCs) of HDM+ allergic rhinitis (AR) patients or healthy
429 controls. **A:** Stimulation of PBMCs of monosensitized HDM⁺AR patients and control
430 subject with house dust mite (HDM) (n = 6); **B:** Stimulation of PBMC of
431 monosensitized HDM⁺AR patients (n = 8) and control subjects (n = 5) with *Artemisia*
432 *annua* (*A. annua*); **C:** The positive ratio of cytokine produced by PBMCs of
433 monosensitized HDM⁺ allergic rhinitis or controls upon HDM and *A. annua*
434 stimulation. **D:** Concentrations of IL-17, IL-5 and IFN- γ released in PBMCs
435 stimulated with synthesized tropomyosin peptides of *A. annua* and Der p10 (n = 4).

A **B**

The prevalence of inhalant allergens in polysensitized HDM⁺ AR patients

C

The positive rate of HDM⁺ AR patients in outpatients