

# **Der p 1-based Immunotoxin as Potential Tool for the Treatment of Dust Mite respiratory Allergy**

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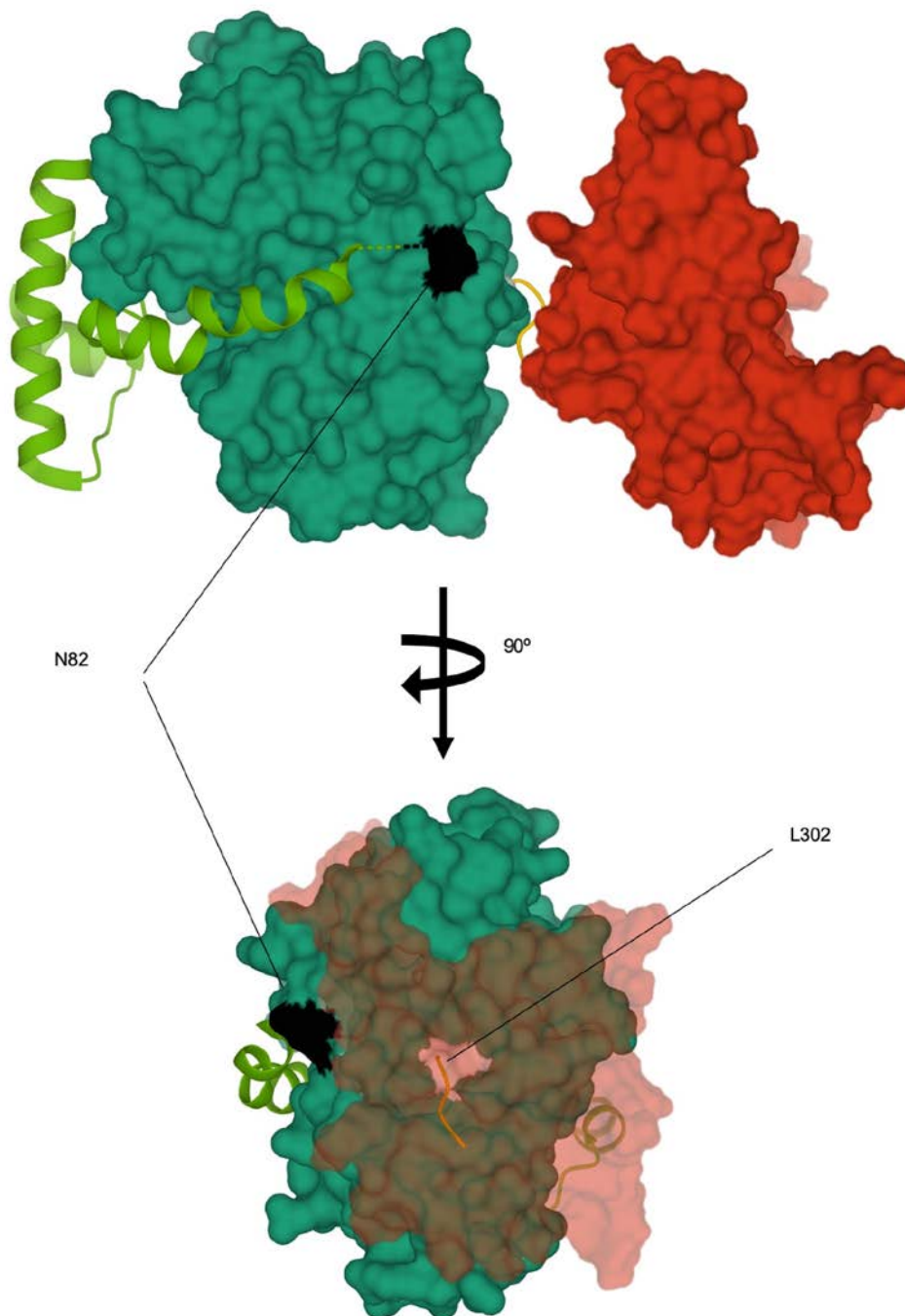


Figure S1

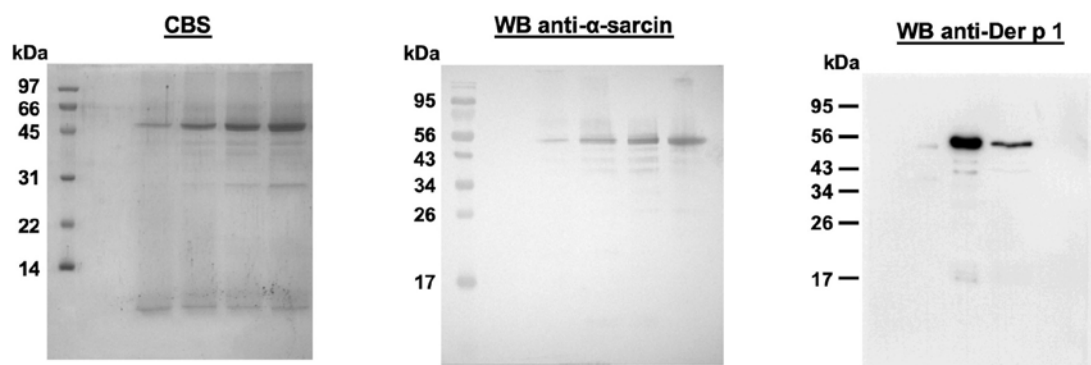


Figure S2

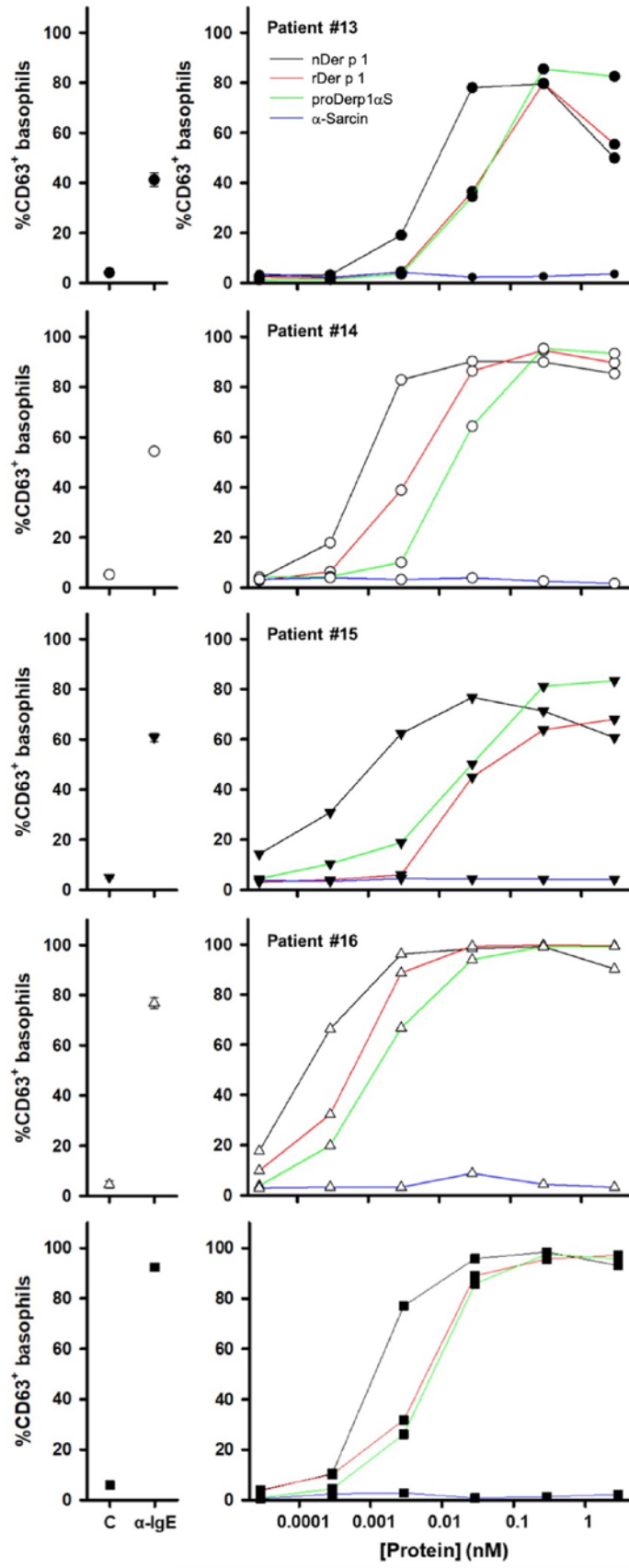


Figure S3

Serum number	slgE to <i>D. pteronyssinus</i> (kU/L)
1*	57.5
2	47.7
3	36.2
4	76.7
5*	100
6*	84.3
7	51.4
8	36.2
9	47.4
10	100
11	100
12*	30.3
13*/**	>100
14**	>100
15**	27.3
16**	51,1
17**	>100
C1*	<0.35
C2**	<0.35

Table S1

## Supplementary Figure Legends

### **Figure S1. Schematic representation of proDerp1 $\alpha$ S immunotoxin structure.**

Three-dimensional conformational representation of proDerp1 $\alpha$ S construct built in accordance to crystal structures of Der p 1 pro form (PDB code: 1XKG; in turquoise) and  $\alpha$ -sarcin (PDB code: 1DE3; in red). The green ribbon diagram represents Der p 1 N-terminal pro-peptide while the glycine-glycine-arginine linker (GGR) appears in yellow. The structures of mature Der p 1 and  $\alpha$ -sarcin are shown as molecular surface representations. The Der p 1 mature N- and C-terminal residues (N82, in black, and L302, in white) are also indicated.

### **Figure S2. Coomassie blue Staining (CBS) SDS-PAGE and Western Blot analysis of purified proDerp1 $\alpha$ S immunotoxin.**

Full-length gels from Figure 1B are displayed. CBS SDS-PAGE (left) and Western blot analysis using rabbit anti- $\alpha$ -sarcin (middle) or anti-Der p 1 antisera (right). CBS protein molecular weight standards correspond to Bio-Rad Unstained SDS-PAGE low range Standards; while those shown in the blots are prestained Bio-Rad Precision Plus protein molecular weight standards. Images correspond to full-length gels and blots acquired and analysed using the Gel Doc XR Imaging System and Quantity One 1-D analysis software (BioRad) (left/center) or ChemiDoc-It (UVP) and VisionWorks LS (right).

**Figure S3. Individual basophil degranulation profile for each patient.** Basophil activation test dose dependant profiles for nDer p 1, rproDer p 1, proDerp1 $\alpha$ S and  $\alpha$ -sarcin performed with basophils from Der p 1-allergic patients (n=5; #13, 14, 15, 16 and 17). Non-stimulated (C) and  $\alpha$ -IgE antibody stimulated samples ( $\alpha$ -IgE) were included as controls in each patient test (left column plots). Overlapped and analysed data are displayed in Figure 6.

**Table S1. Levels of specific IgE to *D. pteronyssinus* crude extract in sera from donors.** Specific IgE (sIgE) level from allergic patients (#1-17) and non-allergic subjects (C1 and C2) was determined by ImmunoCAP-FEIA in serum samples. Single asterisk indicates sera used in *in vitro* humRBL-2H3 degranulation assays and viability assays. Black arrows indicate for *in vitro* humRBL-2H3 degranulation and viability assays. Double asterisk indicates selected patients for BAT and basophils cytotoxicity assays.