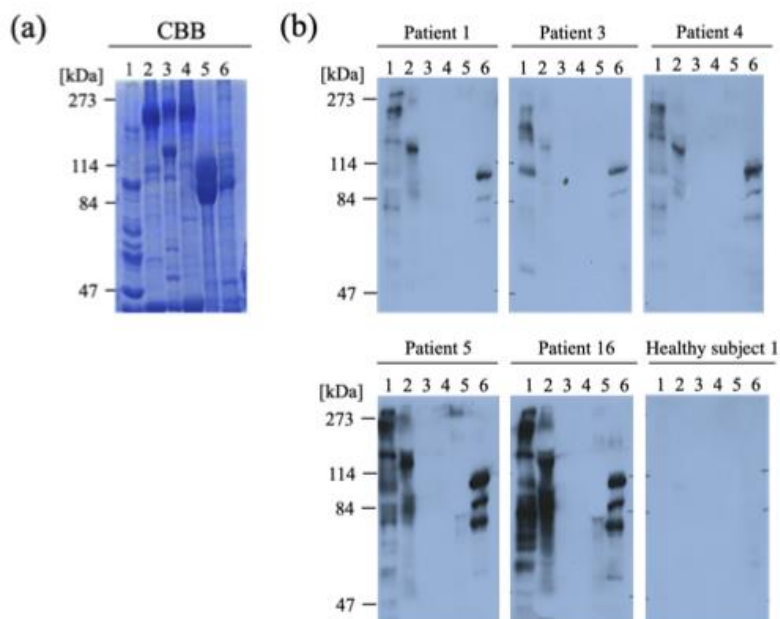


## SUPPLEMENTARY MATERIAL

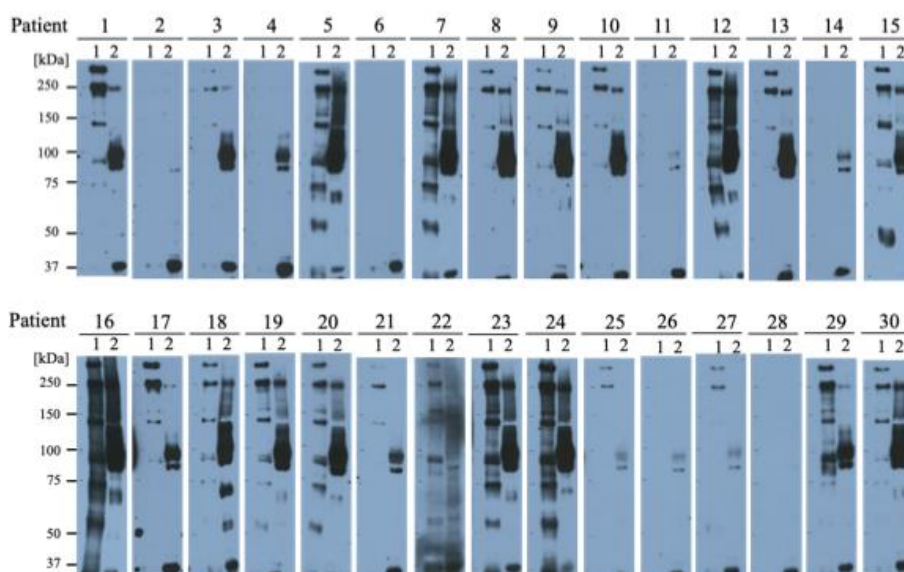
## SUPPLEMENTARY MATERIAL

Supplementary Figure 1. IgE immunoblotting of beef, flounder meat and roe. (a) Gel stained with Coomassie Brilliant Blue (CBB); (b) IgE immunoblotting with sera of patients. Lane 1, water-soluble beef fraction; lane 2, water-insoluble beef fraction; lane 3, water-soluble flounder meat fraction; lane 4, water-insoluble flounder meat fraction; lane 5, water-soluble flounder roe fraction; lane 6, water-insoluble flounder roe fraction. Beef, meat and roe protein fractions were used at 25 mg/lane.



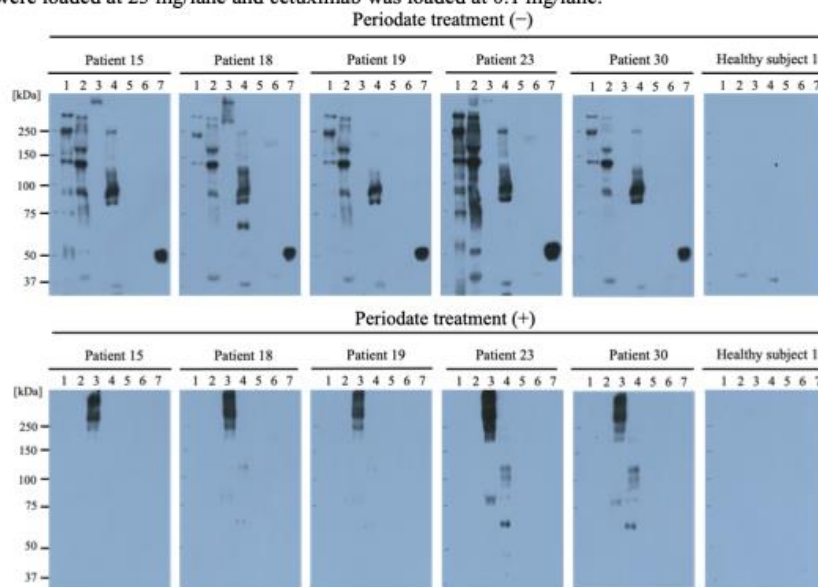
**SUPPLEMENTARY MATERIAL**

Supplementary Figure 2. IgE immunoblotting of water-soluble beef fraction and water-insoluble flounder roe fraction. Lane 1, water-soluble beef fraction (30 µg/lane); lane 2, water-insoluble flounder roe fraction (30 µg/lane). IgE binding to water-soluble beef fraction and water-insoluble flounder roe fraction were analyzed by immunoblotting using sera from all 30 patients.



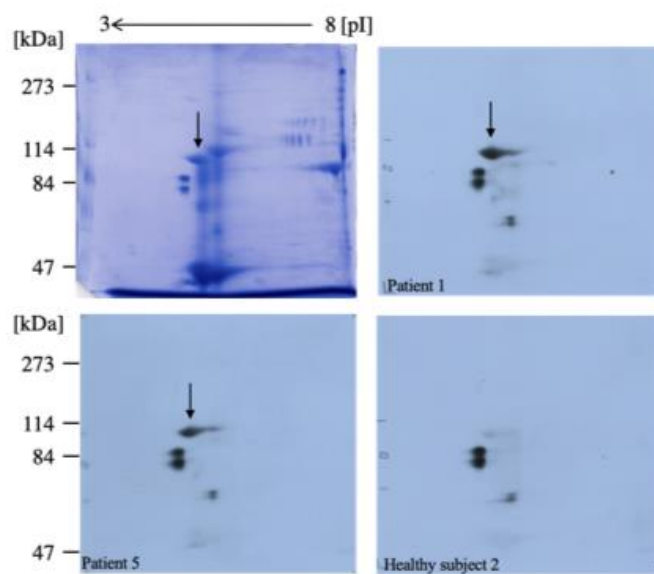
### SUPPLEMENTARY MATERIAL

Supplementary Figure 3. Detection of the carbohydrate moiety of water-insoluble flounder roe allergens. Water-soluble and water-insoluble protein fractions of beef, flounder roe, and flounder meat as well as cetuximab were electrophoresed and transferred to PVDF membranes. The membranes were incubated with a solution containing 50 mM sodium acetate (pH 4.5) and 20 mM sodium periodate for 1 h in the dark and further incubated with patients' sera as previously described [6]. Lane 1, water-soluble beef fraction; lane 2, water-insoluble beef fraction; lane 3, water-soluble flounder roe fraction; lane 4, water-insoluble flounder roe fraction; lane 5, water-soluble flounder meat fraction; lane 6, water-insoluble flounder meat fraction; lane 7, cetuximab. All beef, meat and roe fractions were loaded at 25 mg/lane and cetuximab was loaded at 0.1 mg/lane.



**SUPPLEMENTARY MATERIAL**

Supplementary Figure 4. IgE immunoblotting upon 2D-PAGE of water-insoluble flounder roe proteins. Water-insoluble flounder roe protein fraction (200  $\mu$ g) was separated by 2D-PAGE and blotted with the sera of the patients with red meat allergy (patients 1, 5) and healthy subject 2.



Accepted

## SUPPLEMENTARY MATERIAL

Supplementary Figure 5. Nucleotide sequence of the cDNA clone obtained using the 3'-RACE and 5'-RACE methods and its putative amino acid sequence. The signal sequence and the N-terminal amino acid sequence are underlined with red single and blue double lines, correspondingly.

a ggg tca gcc acc tca cac cag agc tgg ttt gta gat tgc tgg gct tcc	49	
Gly Ser Ala Thr Ser His Gln Ser Trp Phe Val Asp Cys Trp Ala Ser		
1 5 10 15		
agt gcc acg ctc taa aag tgg ttt gga ctc ctg tga ttt ttt gtc ttt	97	
Ser Ala Thr Leu Lys Trp Phe Gly Leu Leu Phe Phe Val Phe		
20 25 30		
gta cac aac aac atg agg ggg oct gag cac att ttg tta tgg acc ttc	145	
Val His Asn Asn Met Arg Gly Pro Glu His Ile Leu Leu Trp Thr Phe		
35 40 45		
atg att gct gca gtt gac acc ttt gct caa cag agg ctg aat ctg aag	193	
<u>Met Ile Ala Ala Val Asp Thr Phe Ala</u> Gln Pro Arg Leu Asn Leu Lys		
50 55 60		
cac aat tgg cag tca gcc agc ggt tta agg tcc gac tgt gca ggg aat	241	
His Asn Ser Gln Ser Gly Ser Gly Leu Arg Ser Asp Cys Ala Gly Asn		
65 70 75 80		
ctg atg aga gtc tcc ttg gac aag gct ctg . . . . .		
<u>Leu Met Arg Val Ser Leu Asp Lys Ala Leu</u>		
85 90		
ggc gac gct gat gac tcc tgc agc gcc cag tgt gtg aat cct aca ggc	2737	
Gly Asp Ala Asp Asp Ser Cys Arg Gly Gln Cys Val Asn Pro Thr Gly		
900 905 910		
atg aag ccc tac agc caa caa ggg gtt aaa aga gag cga aga agc aca	2785	
Met Lys Pro Tyr Ser Gln Gln Gly Val Lys Arg Glu Arg Arg Ser Thr		
915 920 925		
aac tcc agc aac caa agg cag ctc tct tct gga cca atc ctg tta ctc	2833	
Asn Ser Ser Asn Gln Arg Gln Leu Ser Ser Gly Pro Ile Leu Leu Leu		
930 935 940		
agt caa act tct gaa taa aaa aaa att ctt aaa atg aaa aaa aaa	2881	
Ser Gln Thr Ser Glu Lys Lys Ile Leu Lys Met Lys Lys Lys Lys		
945 950 955 960		
aaa aaa aaa aaa aaa aaa aaa aaa aaa agg atc cgg tac ctc	2929	
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Arg Ile Arg Tyr Leu		
965 970 975		
tag atc aga	2938	
Ile Arg		

— Signal sequence  
— N-terminal amino acid sequence