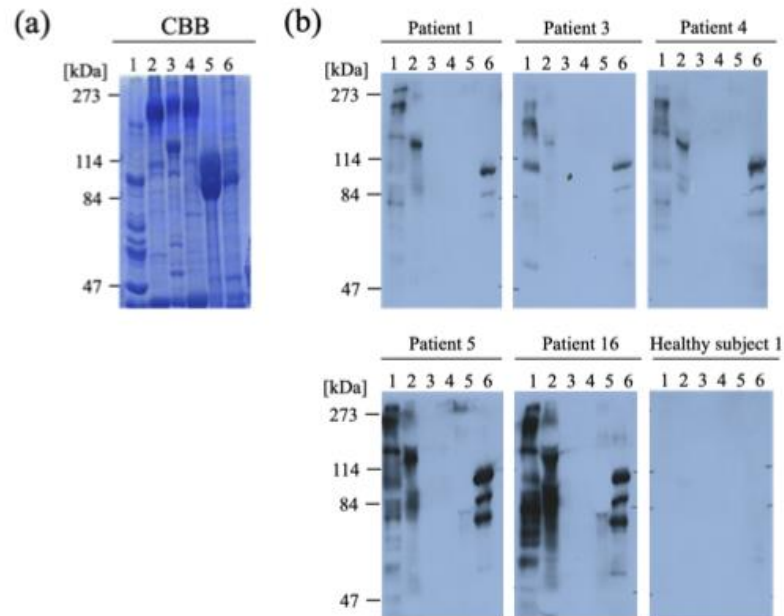
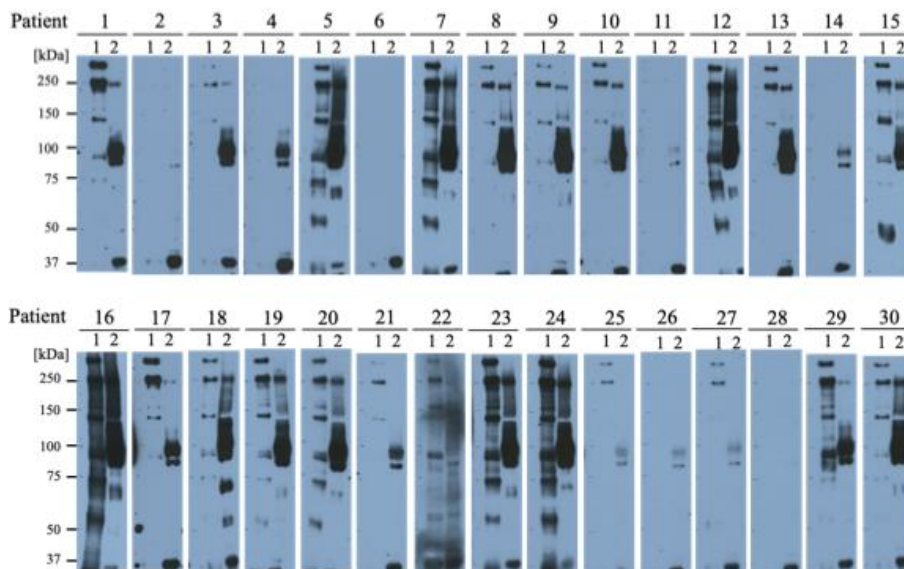


SUPPLEMENTARY MATERIAL

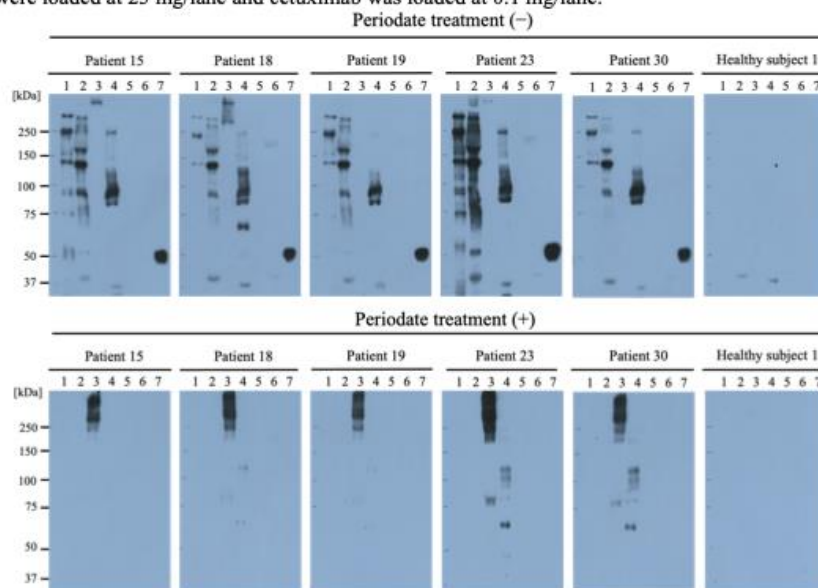
Supplementary Figure 1. IgE immunoblotting of beef, flounder meat and roe. (a) Gel stained with Coomassie Brilliant Blue (CBB); (b) IgE immunoblotting with sera of patients. Lane 1, water-soluble beef fraction; lane 2, water-insoluble beef fraction; lane 3, water-soluble flounder meat fraction; lane 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.



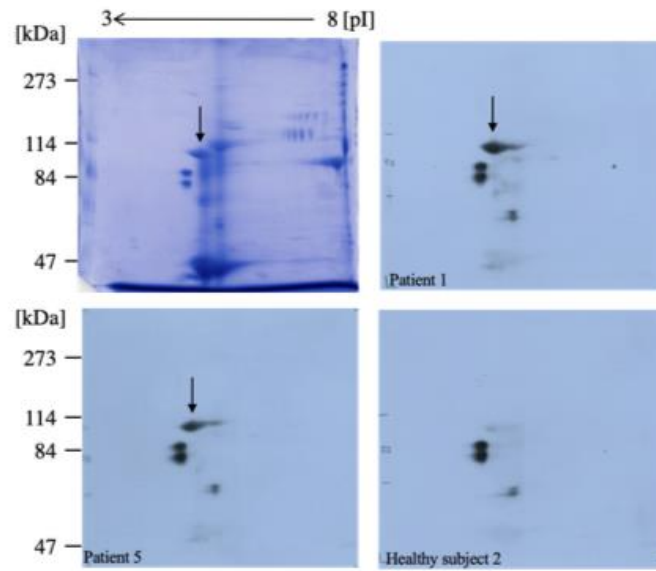
Supplementary Figure 2. IgE immunoblotting of water-soluble beef fraction and water-insoluble flounder roe fraction. Lane 1, water-soluble beef fraction (30 µg/lane); lane 2, water-insoluble flounder roe fraction (30 µg/lane). IgE binding to water-soluble beef fraction and water-insoluble flounder roe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



Supplementary 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 Figure 4. IgE immunoblotting upon 2D-PAGE of water-insoluble flounder roe proteins. Water-insoluble flounder roe protein fraction (200 μ 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.



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.

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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
Met Ile Ala Ala Val Asp Thr Phe Ala Gln Pro Arg Leu Asn Leu Lys
50 55 60

cac aat tgg cag tca ggc 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 * * * * *
Leu Met Arg Val Ser Leu Asp Lys Ala Leu
85 90

ggc gac gct gat gac tcc tgc agc ggc 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 atg 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

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— Signal sequence
— N-terminal amino acid sequence