Receptor destroying enzyme (RDE) from *Vibrio cholerae* modulates IgE activity and reduces the initiation of anaphylaxis

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Running title: RDE modulates IgE to not induce anaphylaxis

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List of materials

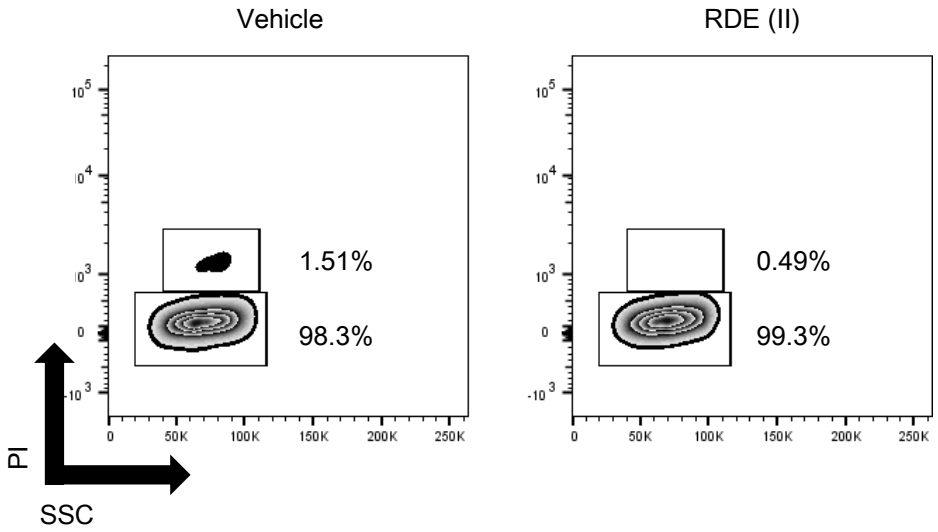

Figure S1.
Confirmation of the toxicity of RDE (II) for BMMCs by PI staining. BMMCs were incubated in untreated IgE (Vehicle) or RDE (II)-treated IgE for 2 h at 37°C. Dead cells were detected by propidium iodide (PI). Data are representative of at least two independent experiments.

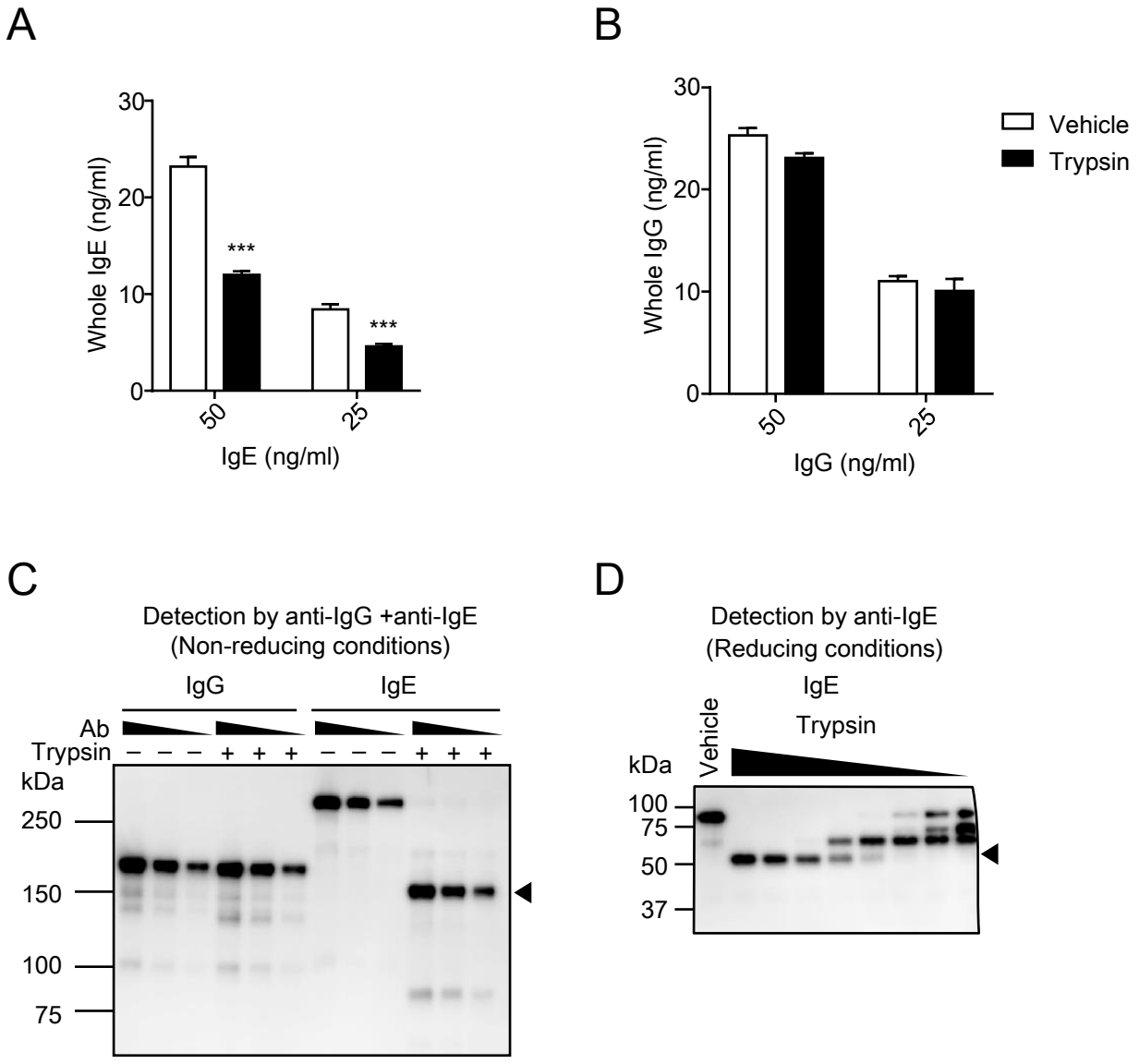

Figure S2.
Trypsin also changes the structure of IgE and reduces its binding activity to anti-ε chain, but not that of IgG. (A and B) Fifty or twenty-five ng/ml of purified IgE (C38-2) or IgG (15H6) were incubated with 10 μg/ml of trypsin at 37°C overnight (12-20 h). The level of IgE (A) and IgG (B) were measured by quantitative ELISA. (C) Serially diluted purified IgG and IgE (390 ng~, 2-fold dilutions) were treated with 10 μg/ml trypsin at 37°C overnight (12-20 h). Then, they were blotted under non-reducing conditions. The specimens were analyzed with HRP-conjugated anti-mouse IgG and IgE. (D) Purified IgE were treated with serially diluted trypsin (630 μg/ml~, 3-fold dilutions) overnight (12-20 h). They were blotted under reducing conditions. The specimen was analyzed with HRP-conjugated anti-mouse IgE. Data are representative of at least two independent experiments and indicate the mean ± standard deviation. ***$P<0.001$ (Student's *t*-test).

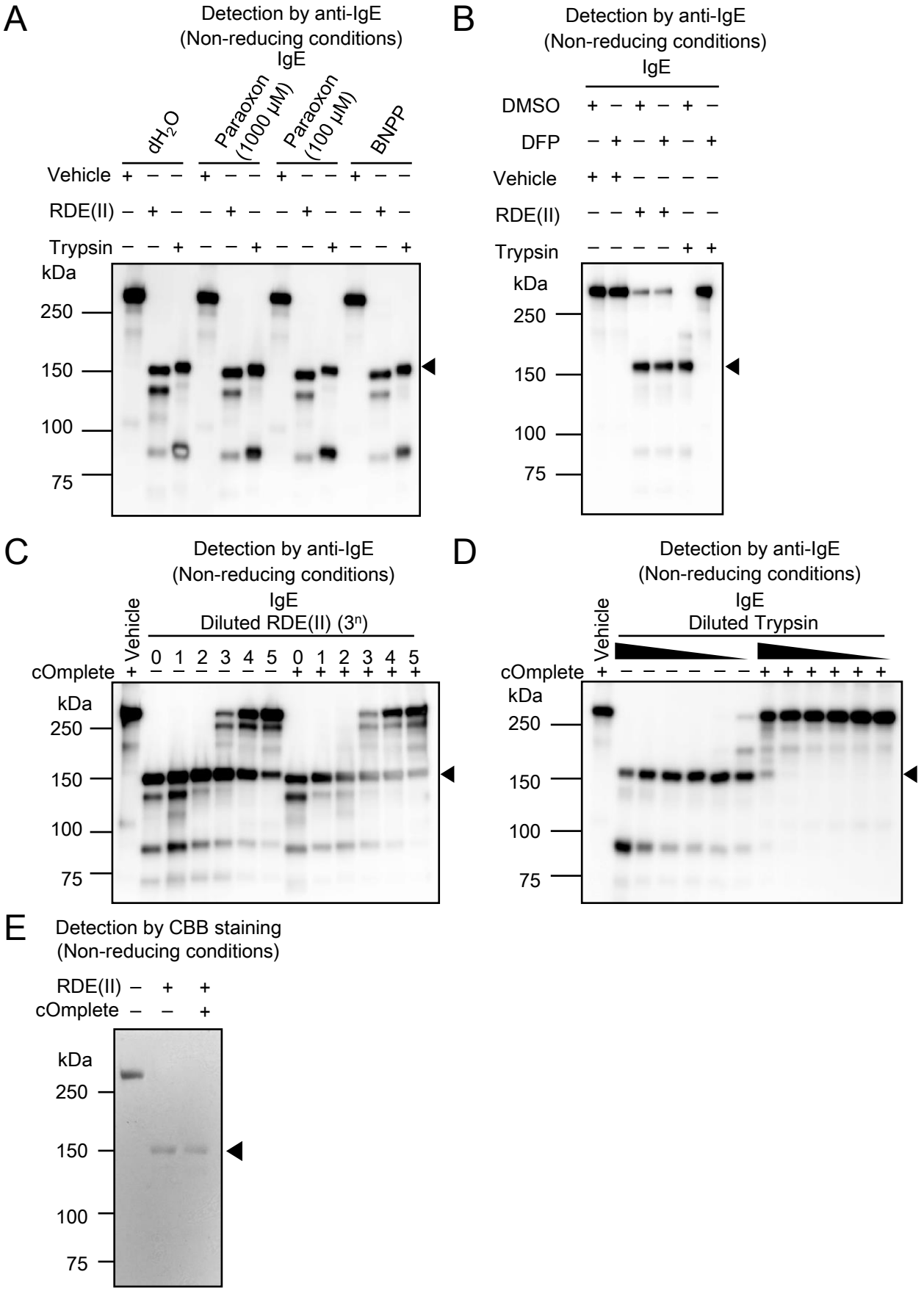
A
Detection by anti-IgE
(Non-reducing conditions)
IgE
dH2O
Paraoxon (1000 μM)
Paraoxon (100 μM)
BNPP
Vehicle + – – + – – + – – + – –
RDE(II) – + – – + – – + – – + –
Trypsin – – + – – + – – + – – +
kDa
250
150
100
75
B
Detection by anti-IgE
(Non-reducing conditions)
IgE
DMSO + – + – + –
DFP – + – + – +
Vehicle + + – – – –
RDE(II) – – + + – –
Trypsin – – – – + +
kDa
250
150
100
75
C
Detection by anti-IgE
(Non-reducing conditions)
IgE
Vehicle
Diluted RDE(II) (3n)
0 1 2 3 4 5 0 1 2 3 4 5
cOmplete + – – – – – – + + + + + +
kDa
250
150
100
75
D
Detection by anti-IgE
(Non-reducing conditions)
IgE
Vehicle
Diluted Trypsin
cOmplete + – – – – – – + + + + + +
kDa
250
150
100
75
E
Detection by CBB staining
(Non-reducing conditions)
RDE(II) – + +
cOmplete – – +
kDa
250
150
100
75

Figure S3.
Protease inhibitors cannot inactivate RDE (II) to modulate IgE. (A and B) RDE (II) and trypsin were pre-treated with paraoxon as indicated, 1000 μM BNPP (A), and 1000 μM DFP (B) for 30 min. Purified IgE (C38-2) were treated with RDE (II) overnight (12-20 h). They were blotted under non-reducing conditions. (C-D) Purified IgE were treated with serially 3-fold diluted RDE (II) (C) or trypsin (630 μg/ml~) (D) in presence of protease inhibitor (cOmplete) overnight (12-20h). They were blotted under non-reducing conditions. The specimens were analyzed with HRP-conjugated goat mouse anti-IgE. (E) Purified IgE were treated with RDE (II) in the presence of protease inhibitor (cOmplete) overnight (12-20h). The specimens were analyzed with CBB staining. Data are representative of two independent experiments.

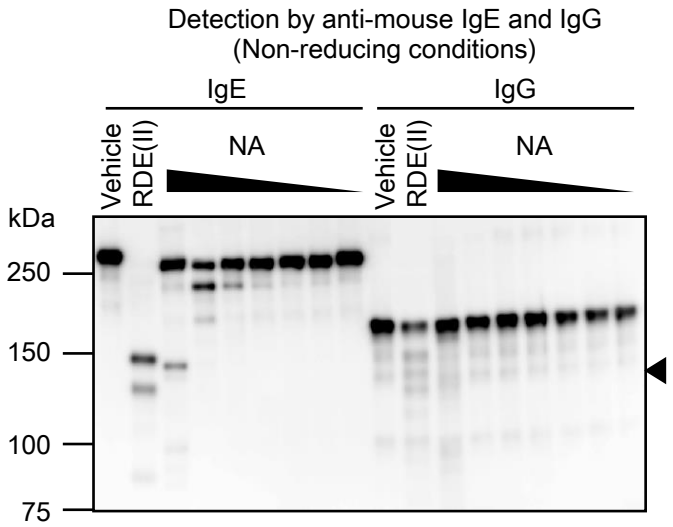

Figure S4.
NA (sialidase) cannot modulate IgE as does RDE (II). Purified IgE (C38-2) or IgG (15H6) were treated with serially diluted NA from *Arthobacter ureafaciens* (0.3 U~) in 50 mM sodium acetate buffer (pH 5.5). They were blotted under non-reducing conditions. Data are representative of two independent experiments.

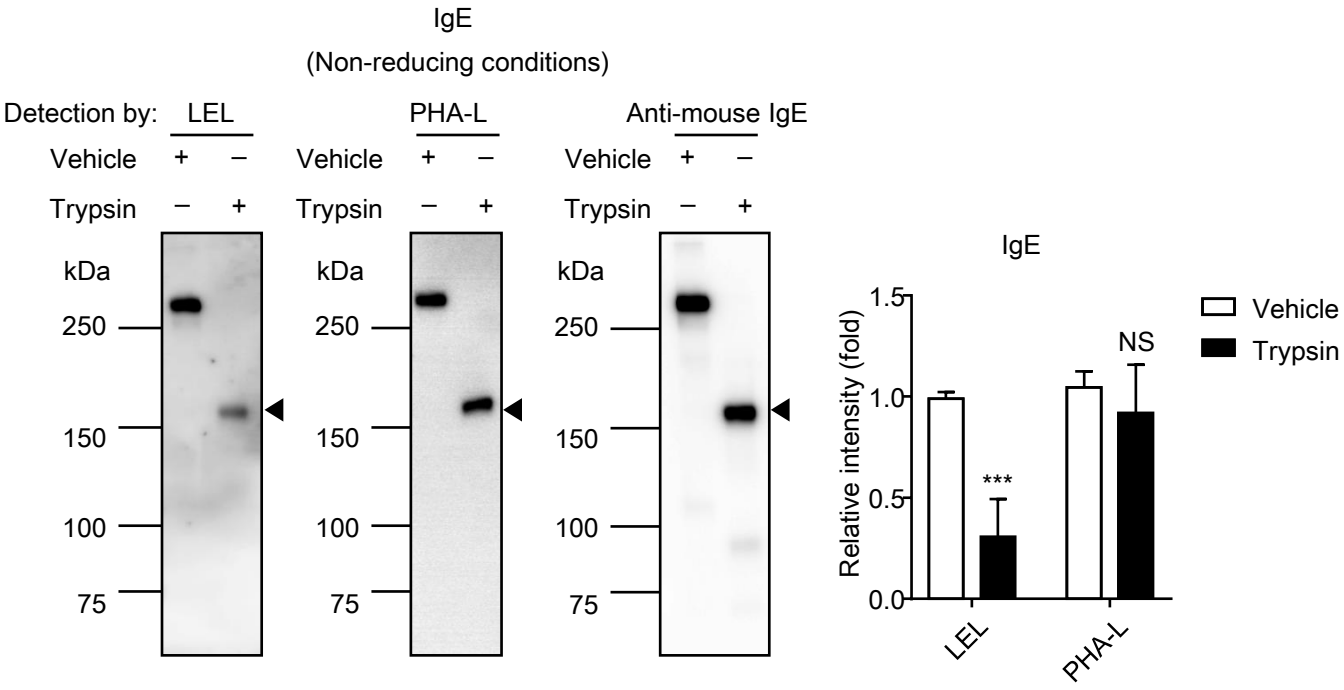

Figure S5.
Trypsin reduced the binding level of LEL, not PHA-L, to IgE. Purified IgE (C38-2) was treated with 10 μg/ml of trypsin overnight (12-20 h). They were blotted under non-reducing conditions. The specimens were analyzed with biotin-conjugated LEL (left panel) or PHA-L (middle panel), followed by incubation with HRP-conjugated streptavidin. Internal control was detected with HRP-conjugated anti-mouse IgE (right panel). The signal intensity of LEL and PHA-L was normalized by the internal control (bar graph). Data are representative of two independent experiments and indicate the mean ± standard deviation.

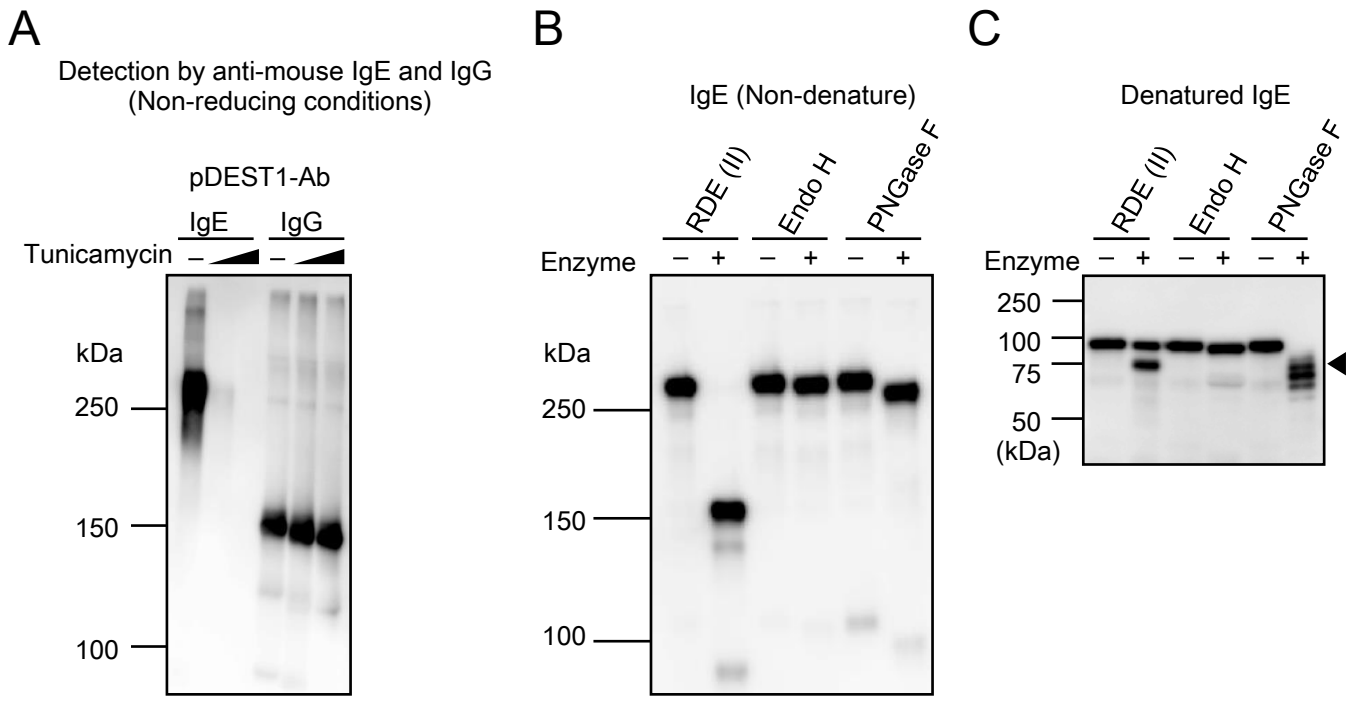

Figure S6.
RDE (II) is a potent enzyme and modulates IgE, compared with PNGase F and Endo H.
(A) HEK293T cells were transfected with anti-HA IgG or anti-HA IgE either with or without tunicamycin. Forty hours later, the supernatants were obtained and blotted by western blotting under non-reducing conditions, followed by incubation with HRP-conjugated anti-mouse IgE and IgG. (B and C) Purified IgE (C38-2) under non-denaturing (B) or denaturing by 0.5% SDS and 40 mM DTT (C) were treated with RDE (II), 10 U Endo H, and 10 U PNGase F at 37℃ overnight (12-20 h). They were blotted, followed by incubation with HRP-conjugated anti-mouse IgE and IgG. Data are representative of two independent experiments.

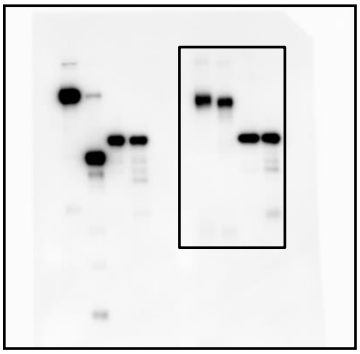

Fig.1C

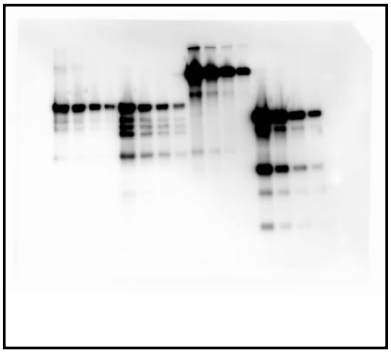

Fig.2A

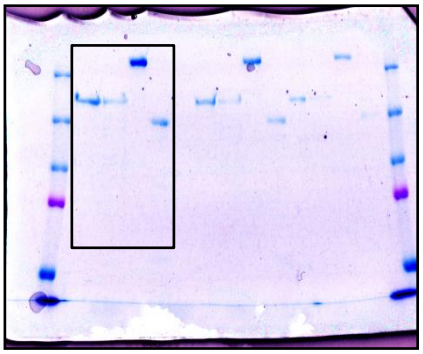

Fig.2B

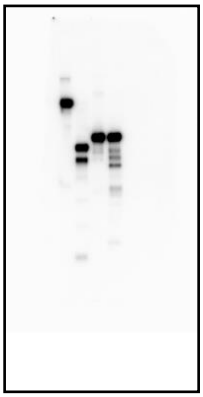

Fig.2C

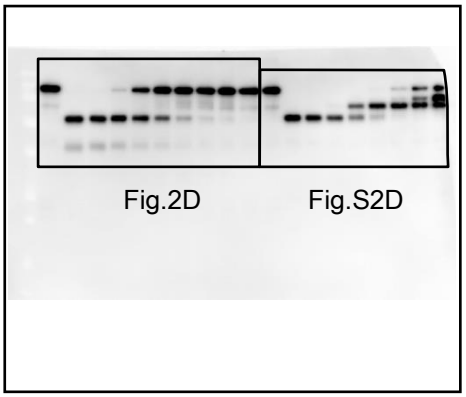

Fig.2D & S2D

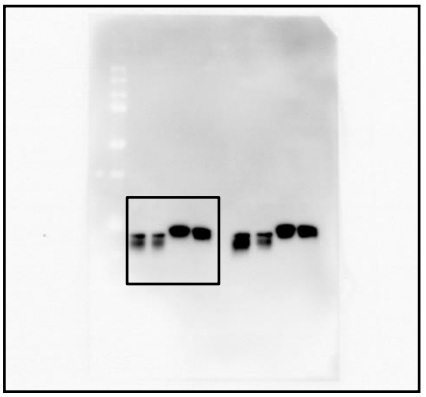

Fig.2E

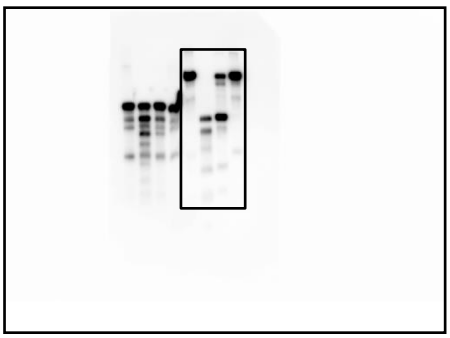

Fig.5B

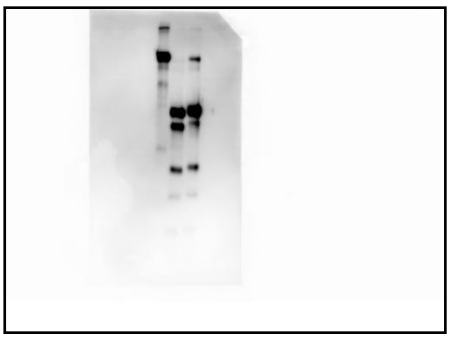

Fig.5C

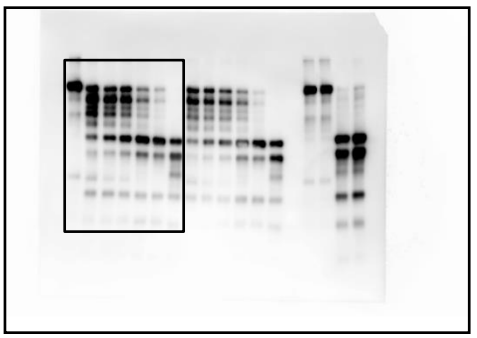

Fig.5D

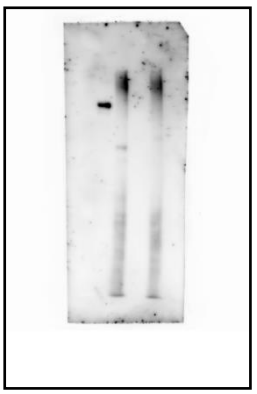

Fig.6D

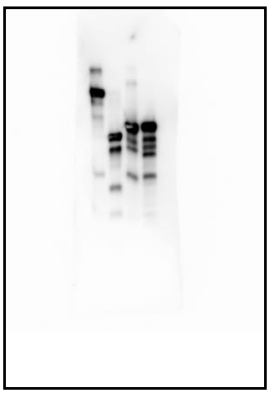

Fig.6E

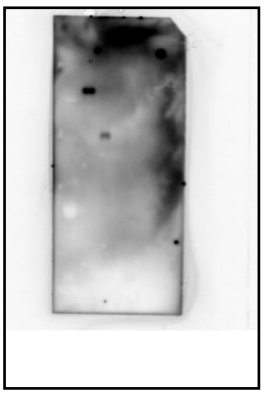

Fig.6F

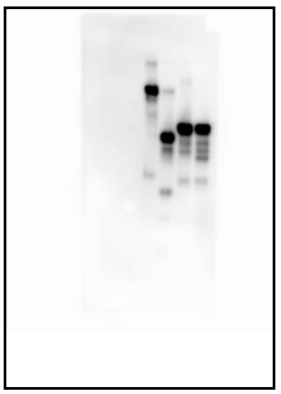

Fig.6G

Figure S7. Full-length blots from the main figures. Some cropped areas used in the article are indicated in black boxes.

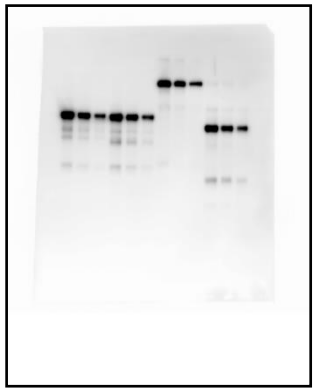

Fig. S2C

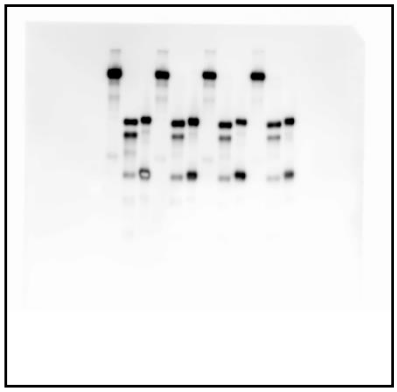

Fig. S3A

Fig. S3B

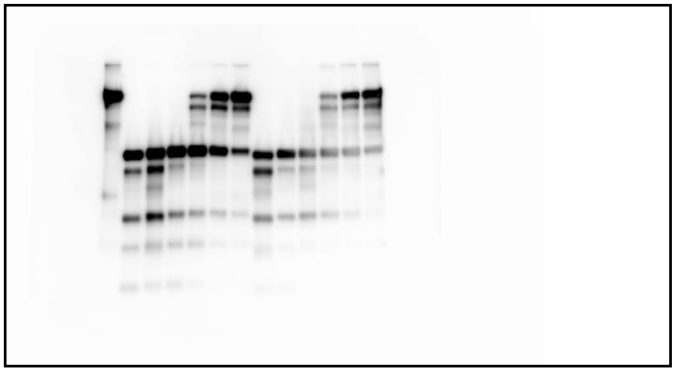

Fig. S3C

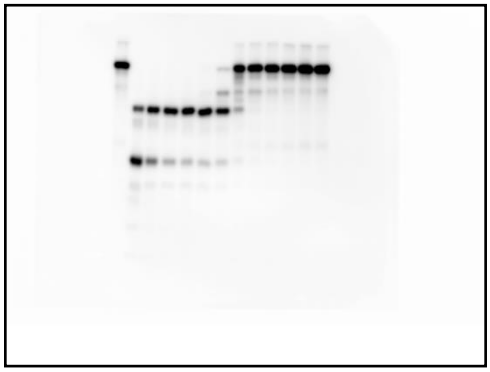

Fig. S3D

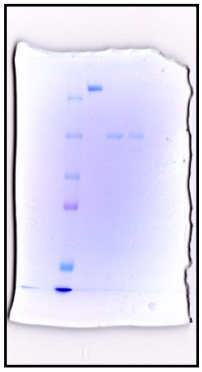

Fig. S3E

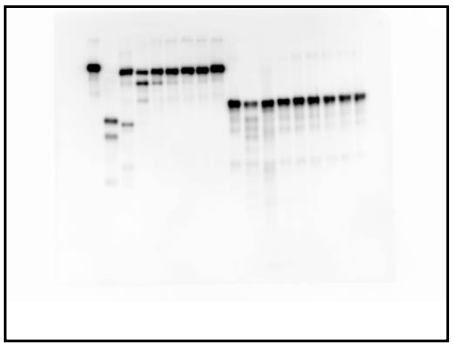

Fig. S4

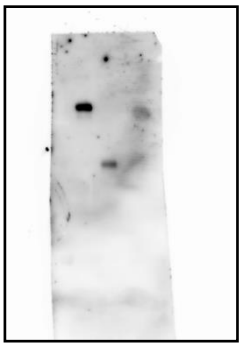

Fig.S5 (LEL)

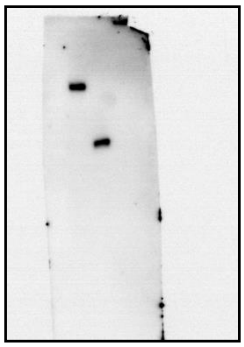

Fig.S5 (PHA-L)

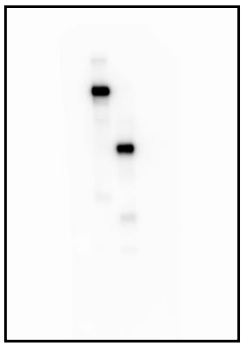

Fig.S5 (IgE)

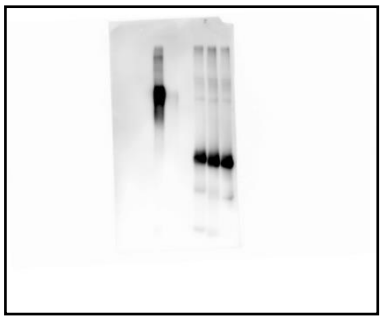

Fig. S6A

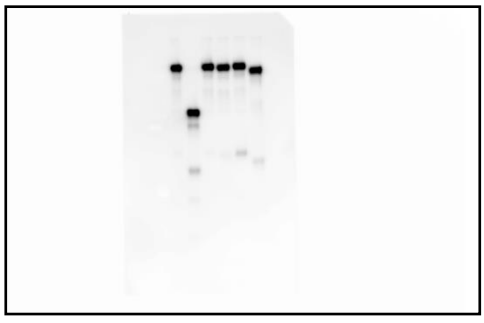

Fig. S6B

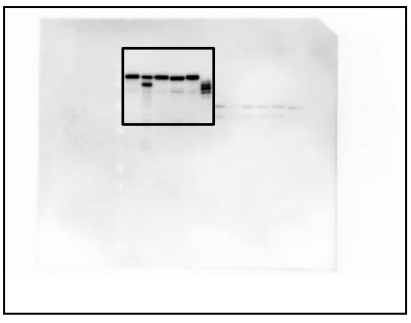

Fig. S6C

Figure S8. Full-length blots from the supplementary figures. Some cropped areas used in the article are indicated in black boxes.

Name	*P* value	*T* value	Rough specificity[2]
PHAL	0.004292593	15.21382552	GlcNAcb1-6Man (Tetraantenna)
RCA120	0.006710645	-12.14572601	bGal
LEL	0.009109669	10.40556381	Polylactosamine, (GlcNAc)n
ACA	0.009448592	-10.21460928	Galb1-3GalNAc (T)
rCGL2	0.010664924	-9.60562312	GalNAca1-3Gal (A), PolyLacNAc
ASA	0.010806471	9.541481089	Galb1-4GlcNAcb1-2Man
STL	0.013035735	8.672686436	Polylactosamine, (GlcNAc)n
UDA	0.024121881	6.321569548	(GlcNAc)n
CSA	0.036796322	5.068135814	Rhamnose, Gala1-4Gal
rBanana	0.041390229	4.761392433	Mana1-2Mana1-3(6)Man
SBA	0.043098637	4.659785585	a,bGalNAc (A, Tn, LacDiNAc)
DBAI	0.044635839	4.573284989	High-man
rPPL	0.049888162	4.30785636	a,bGalNAc (A, Tn, LacDiNAc)
MAL	0.049938162	4.305527838	a2-3Sia
rGal9C	0.066443378	-3.68342103	PolyLacNAc, Branched LacNAc
AOL	0.069215478	3.600772094	a1-2Fuc (H), a1-3Fuc (Lex), a1-3Fuc (Lea)
MCA	0.073581797	3.479858593	a1-2Fuc
BPL	0.122407202	2.588831937	Galb1-3GlcNAc(GalNAc), a/bGalNAc
rCNL	0.124400591	2.563534649	a,bGalNAc (A, Tn, LacDiNAc)
GNA	0.134623911	2.442168276	Mana1-3Man, Mana1-6Man
VVA I	0.135176376	2.435977443	GalNAcb1-3(4)Gal
HHL	0.137744253	2.40765741	Mana1-3Man, Mana1-7Man
CCA	0.140204199	2.381207142	Galactosylated N-glycans up to triantenna
rACG	0.155057486	-2.234109074	a2-3Sia
NPA	0.168641861	2.115601255	Mana1-3Man
SSA	0.177972501	-2.041490805	a2-6Sia
PTLI	0.179425875	2.030420999	aGalNAc (A, Tn)
rPTL	0.184949542	-1.989429308	a1-6Fuc
rBC2LCN	0.199142655	-1.891247462	Fuc a1-2Galb1-3GlcNAc (GalNAc)
WFA	0.211422499	1.813551519	Terminal GalNAc, LacDiNAc
rDiscoidin II	0.244081441	1.632940805	LacNAc, Galb1-3GalNAc (T), GalNAc (Tn)
VVA	0.25554222	-1.576861349	a,bGalNAc (A, Tn, LacDiNAc)
PHAE	0.259209119	1.559596265	bisecting GlcNAc
rLSLN	0.281031687	-1.462895041	LacNAc, polylactosamine
rCGL3	0.282482338	-1.456804715	LacDiNAc
HEA	0.291057767	-1.421584106	Galb1-3GalNAc (T)
GSLII	0.296997055	-1.397939483	GlcNAcb1-4Man
GSLIA4	0.313779867	1.334162658	aGalNAc (A, Tn)
rF17AG	0.348384729	-1.214848863	GlcNAc
ConA	0.353551629	1.198252556	M3, Mana1-2Mana1-3(Mana1-6)Man, GlcNAcb1-2Mana1-3(Mana1-6)Man
TJAI	0.363480022	-1.167148479	a2-6Sia
rGal8N	0.384245748	-1.105172904	a2-3Sia
UEAI	0.392245605	1.082316328	a1-2Fuc
rRSIIL	0.397261466	-1.068253674	a1-2Fuc (H), a1-3Fuc (Lex), a1-3Fuc (Lea)
DBA	0.39826288	-1.065470098	a,bGalNAc (A, Tn, LacDiNAc)
FLAG-EW29Ch	0.401643298	1.056131613	Gal
rMOA	0.401701184	-1.05597247	aGal (B)
MAH	0.404835483	-1.047393812	a2-3Sia
rSRL	0.411044018	-1.030618029	Core1,3, agalacto N-glycan
EEL	0.412476337	1.026787889	aGal (B)
rOrysata	0.414625085	1.021069556	Mana1-3Man, Highman, biantenna
rGRFT	0.421769884	1.002288528	Man
rMalectin	0.422649731	1	Glca1-2Glc
PNA	0.4266081	-0.989768317	Galb1-3GalNAc (T)
DSA	0.434556488	-0.969532572	GlcNAcb1-6Man (Tetraantenna)
ACG	0.436244966	-0.965285662	a2-3Sia
HPA	0.43993936	-0.956055051	aGalNAc (A, Tn)
rGal3C	0.452603739	0.925032484	LacNAc, polylactosamine
GSLIB4	0.454454584	0.920576277	aGal (B)
rPSL1a	0.458773585	-0.910251825	a2-6Sia
rXCL	0.474716039	-0.873005207	Core1,3, agalacto N-glycan
MPA	0.480039208	-0.860857062	Galb1-3GalNAc (T), GalNAca (Tn)
rAOL	0.504451872	-0.806846227	a1-2Fuc (H), a1-3Fuc (Lex), a1-3Fuc (Lea)
LTL	0.510705102	0.793432956	Lex, Ley
rAAL	0.525124622	0.76310763	a1-2Fuc (H), a1-3Fuc (Lex), a1-3Fuc (Lea)
rPAIL	0.526665237	0.759915591	a,bGal, aGalNAc (Tn)
rABA	0.534205258	-0.744421521	Galb1-3GalNAc (T), GlcNAc
LFA	0.559020537	0.694849238	Sia
ADA	0.56815811	-0.677107508	a2-6Sia, Forssman, A, B
rDiscoidin I	0.56976652	-0.674011441	Gal
PVL	0.589971196	0.635769908	Sia, GlcNAc
WGA	0.59409595	-0.7176112	(GlcNAc)n, polySia
TJAII	0.607655183	0.603227196	a1-2Fuc
SNA	0.613488685	-0.592669015	a2-6Sia
rGC2	0.61747264	0.585506572	a1-2Fuc (H), aGalNAc (A), aGal (B)
rC14	0.635018232	-0.5544082	Branched LacNAc
VVAII	0.647175582	-0.533263493	Man, Agalacto
ABA	0.649261583	-0.529666927	Galb1-3GalNAc (T), GlcNAc
rGal9N	0.670483256	0.493573247	GalNAca1-4Gal (A), PolyLacNAc
FLAG-EW29Ch-E20K	0.713541275	-0.422833682	6-sulfo-Gal
Jacalin	0.729787659	-0.396902428	Galb1-3GalNAc (T), GalNAca (Tn)
rBC2LA	0.743586074	-0.375166984	aMan, High-man
rHeltuba	0.744799863	0.373267019	Mana1-3Man
rGal7	0.749989698	-0.365164423	Type1 LacNAc, chondroitin polymer
rCalsepa	0.75933282	0.350661539	Biantenna with bisecting GlcNAc
TxLcI	0.765937307	0.340472448	Galactosylated N-glycans up to triantenna
Heltuba	0.767676507	0.337797661	Mana1-3Man
AAL	0.794410584	0.297093757	a1-2Fuc (H), a1-3Fuc (Lex), a1-4Fuc (Lea)
ECA	0.832577671	-0.240160732	bGal
PWM	0.841900593	0.226434142	(GlcNAc)n
rRSL	0.849848974	-0.214780569	aMan, a1-2Fuc (H), a1-3Fuc (Lex), a1-4Fuc (Lea)
rPAIIL	0.85498368	0.207275097	aMan, a1-2Fuc (H), a1-3Fuc (Lex), a1-4Fuc (Lea)
LCA	0.905171531	-0.134714782	a1-6Fuc up to biantenna
rPALa	0.96422696	0.05062312	Man5, biantenna
PSA	0.973181512	-0.037940716	a1-6Fuc up to biantenna

Table S1. List of lectin probes used for evaluating the reduction in IgE binding levels by lectin micro array analysis.