Supplementary

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" MODIFICATION OF ANTI-GLYCAN IGG AND IGM PROFILES AFFECTED BY ALLERGIC DISEASES"
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Таблица 1S. Results of immunofluorescence determination of AGA-IgM in the serum of patients with bronchial asthma (BA) and healthy donors (HD), the results of the Mann-Whitney test.

N on glycochip	Structure of glycans	Common name	BA (anti-IgM, 532 nm) Median RFU 95% confidence interval	HD (anti-IgM, 532 nm) Median RFU 95% confidence interval	Results of MW-test p-value
Galβ1-3GlcNAcβ (LeC)					
3	Galβ1-3GlcNAcβ-sp	Le^c	14042 (11327,27-20106,01)	13985 (9162,37-16965,21)	P = 0,2454
4	Galβ1-3GlcNAcβ-sp2	Le^c	4592 (3870,94-6583,07)	6508,25 (5459,61-8178,55)	P = 0,0290
10	3'-O-Su-Galβ1-3GlcNAcβ-sp	3'-O-su-Le^c	11130 (7653,72-12929,91)	11011,25 (8823,09-14695,11)	P = 0,8471
16	Galβ1-3(6-O-Su)GlcNAcβ-sp	6-O-su-Le^c	4299,5 (3425,1893-4998,83)	6562 (5553,35-7885,18)	P = 0,0076
17	6-O-Su-Galβ1-3GlcNAcβ-sp	6'-O-su-Le^c	3743,5 (3278,26-5637,11)	5858,75 (4466,69-6889,93)	P = 0,0987
18	6-O-Su-Galβ1-3(6-O-Su)GlcNAcβ-sp2	6,6'-O-su-Le^c	2790 (2475,64-3242,08)	3725,25 (3521,839-4036,59)	P = 0,0012
25	Neu5Acα2-3Galβ1-3GlcNAc-sp	3`-SiaLe^c	18842 (14492-30879)	20316 (16055-24845)	P = 0,8169
31	Neu5Acα2-6Galβ1-3(6-O Su)GlcNAc-sp	6Su-6`-SiaLe^c	2256 (1968-2645)	3783,5 (3259-4538)	**P < 0,0001**
35	Neu5Acα2-3Galβ1-3-(6-Su)GlcNAc-sp	6-Su-3'SiaLe^c	4358 (3799-6374)	7183 (5609-9175)	P = 0,0116
32	GlcNAcβ1-3Galβ1-3GlcNAcβ-sp	GlcNAc3`Le^c	6635 (5371-11269)	9855 (7607-12354)	P = 0,3466
33	Neu5Gcα2-3Galβ1-3-(6-Su)GlcNAc-sp	6-Su-3'NeuGcLe^c	4668	7548	P = 0,2243

			(4286-8358)	(5920-9305)	
43	Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-sp	Le^C3Le^C	10911 (7593-14511)	11988 (9397-15339)	P = 0,6770
44	Galβ1-3GlcNAcα1-6Galβ1-4GlcNAcβ-sp2	Le^Cα6LN	3258 (2329-3939)	5958 (4796-7315)	P = 0,0014
Fucα1-3 GlcNAcβ (LeX) Galβ1-4					
59	Fucα1-3 GlcNAcβ-sp Galβ1-4	LeX	6975 (5054-8489)	9510 (7952-11963)	P = 0,0074
57	Fucα1-3 GlcNAcβ-sp Neu5Acα2-3Galβ1-4	Sia LeX	2477 (2079-2922)	4178 (3809-5195)	**P < 0,0001**
42	Neu5Acα2-6(Fucα1-2)Galβ1-4GlcNAcβ-sp	Sia6Htype2	2172 (1975-2733)	3712 (3352-4357)	**P < 0,0001**
GalNAcα (Tn)					
1	GalNAcα-sp	T_n	3105 (2916,28-4450,3881)	5011,5 (4581,5688-6071,7305)	**P = 0,0006**
22	Neu5Gcα2-6GalNAcα-sp	Neu5Gc-T_n	3399 (2555,59-4128,3026)	4193,75 (3595,29-4711,01)	P = 0,0500
23	Neu5Acβ2-6GalNAcα-sp	β-SiaT_n	2344 (2190-2856)	3644,75 (3152-4339)	**P < 0,0001**
24	Neu5Acα2-3GalNAcα-sp	3-SiaT_n	2244 (1985-2790)	3664 (3249-4087)	**P < 0,0001**
9	Neu5Acα2-6GalNAcα-sp	SiaT_n	3056 (2596,7771-3659,8192)	4419,75 (3789,9173-5151,1)	**P = 0,0006**
38	Neu5Acα2-3 GalNAcα-sp Neu5Acα2-6	3,6-SiaT_n	2579 (2252-2929)	3529 (3053-4032)	P = 0,0008
39	Galβ1-4GlcNAcβ1-3GalNAcα-sp	3-LacNAc-T_n	2495 (2228-3031)	3895 (3641-4875)	**P < 0,0001**
7	Galα1-3GalNAcα-sp	Tαα	2765,5 (2238,1-3059,49)	4456 (3783,8695-4883,9829)	**P < 0,0001**
GalNAcβ					
20	Neu5Acα2-6GalNAcβ-sp		2354,5	3465	**P < 0,0001**

			(2058,61-2758,88)	(3235-4100)	
2	$(GalNAc\beta\text{-}PEG_2)_3\text{-}\beta\text{-}DD$	β-GalNAc cluster	2302,5 (1806,2389-3029,56)	4228,75 (3547,2757-4849,0316)	**P < 0,0001**
34	GalNAcα1-4Galβ1-4GlcNAcβ-sp	GalNAcα4'LN	3344 (3120-3798)	5360 (4524-6278)	**P = 0,0001**
12	GalNAcβ1-3GalNAcβ-sp	di-GalNAcβ (para-Forssman)	3718 (2763,26-4421,62)	5285,25 (4585,83-6133,28)	P = 0,0047
8	Galβ1-3GalNAcβ-sp	$T_{\beta\beta}$	6111 (4419-14272)	6723 (5996-8538)	P = 0,5070
TF					
5	Galβ1-3GalNAcα-sp	TF	2904 (2433,68-3591,1)	4571 (4126,43-5202,1)	**P < 0,0001**
29	Neu5Acα2-3Galβ1-3GalNAcα-sp	3'-sialyl-TF	1993 (1765-2446)	3168 (2789-3466)	**P < 0,0001**
6	Galβ1-3GalNAcα- $O(CH_2)_3NHCO(CH_2)_5NH_2$	TF-long	4293 (3153,66-4693,0671)	5558,75 (4780,57-6788,9312)	**P = 0,0007**
11	3'-O-Su-Galβ1-3GalNAcα-sp	3'-O-su-TF	3171 (2642,153-3696,64)	3844,25 (3484,99-4593,37)	P = 0,0116
15	(6-O-Su-Galβ1)-3GalNAcα-sp	6'-O-su-TF	2584,5 (2157,1591-2981,4514)	3745,5 (3187,07-4548,13)	**P = 0,0005**
36	Galβ1-3 GalNAcα-sp Neu5Acα2-6	6-SiaTF	2605 (2213-2974)	3569 (3070-4330)	**P = 0,0001**
37	Galβ1-3 GalNAcα-sp Neu5Acβ2-6	6-SiaβTF	2946 (2369-3534)	4854 (3918-5531)	**P < 0,0001**
core					
30	GlcNAcβ1-3 GalNAcα-sp GlcNAcβ1-6	core 4	3087 (2421-3688)	4538 (3976-5570)	P = 0,0089
27	GlcNAcβ1-6 GalNAcα-sp Galβ1-3	core 2	2166 (1976-2833)	3675 (3224-4231)	**P < 0,0001**
13	GlcNAcβ1-3GalNAcα-sp	core 3	4750,5 (3585,9343-7471,4458)	6991,25 (6043,52-8574,6571)	P = 0,1335
14	GlcNAcβ1-6GalNAcα-sp	core 6	3884	5816,75	P = 0,0018

			(3227,99-4689,37)	(4831,26-7550,7093)	
Man					
19	Manβ1-4GlcNAc-sp4		1992 (1742-2633)	4071 (3683-5096)	**P < 0,0001**
21	Manα1-4Manβ-sp4		1630 (1458-1867)	3495 (2804-3775)	**P < 0,0001**
Blood group antigens					
47	Fucα1-2 Galβ-sp3 GalNAcα1-3	A_{tri}	3301 (2964-3633)	4599 (3972-4960)	**P = 0,0002**
28	Fucα1-2 Galβ-O$(CH_2)_3$NHCO$(CH_2)_5NH_2$ GalNAcα1-3	A_{tri}-long	2258 (1952-2684)	4021 (3534-4842)	**P < 0,0001**
48	Fucα1-2 Galβ-sp3 Galα1-3	B_{tri}	3481 (3367-4275)	4502 (4002-5394)	**P = 0,0050**
56	Fucα1-4 GlcNAcβ-sp Galβ1-3	LeA	2378 (2151-2723)	3704 (3432-4299)	**P < 0,0001**
26	Fucα1-4 GlcNAcβ-sp 3-O-Su-Galβ1-3	3'-O-su-Le^a	2640 (2382-3344)	3514 (3061-4092)	**P = 0,0273**
41	Fucα1-4 GlcNAcβ-sp Neu5Acα2-3Galβ1-3	$SiaLe^a$	2427 (2071-2918)	3504 (3123-4008)	**P = 0,0001**
58	Fucα1-4 GlcNAcβ-sp Fucα1-2Galβ1-3	LeB	2561 (2368-3189)	4132 (3535-4460)	**P < 0,0001**
40	Fucα1-3 GlcNAcβ-sp Fucα1-2Galβ1-4	Le^y	2559 (2153-2951)	3803 (3545-4488)	**P = 0,0001**
60	Galα1-4Galβ1-4GlcNAcβ-sp3	P1	1999 (1777-2456)	3702 (2981-4352)	**P < 0,0001**
O-Polysaccharides					
45	ZumozanA	ZumozanA	1939	3181	**P < 0,0001**

			(1467-2247)	(2807-3775)	
46	Polysaccharide *E coli*	E coli	2788 (1941-3858)	5298 (4189-6736)	**P = 0,0002**
61	Polysaccharide *Candida albican*	Candida albican	2201 (1883-2805)	3972 (3109-4279)	**P = 0,0001**
62	Polysaccharide *laminarin*	laminarin	2189 (1811-2636)	4056 (3543-4761)	**P = 0,0001**

sp, - $(CH_2)_3NH_2$, - $(CH_2)_2NH_2$ or -$NHCOCH_2NH_2$; GlcA, glucuronic acid; Sia, Neu5Ac-N-acetyl neuraminic acid; Su, sulfate; OS, oligosaccharide; P, phosphate.

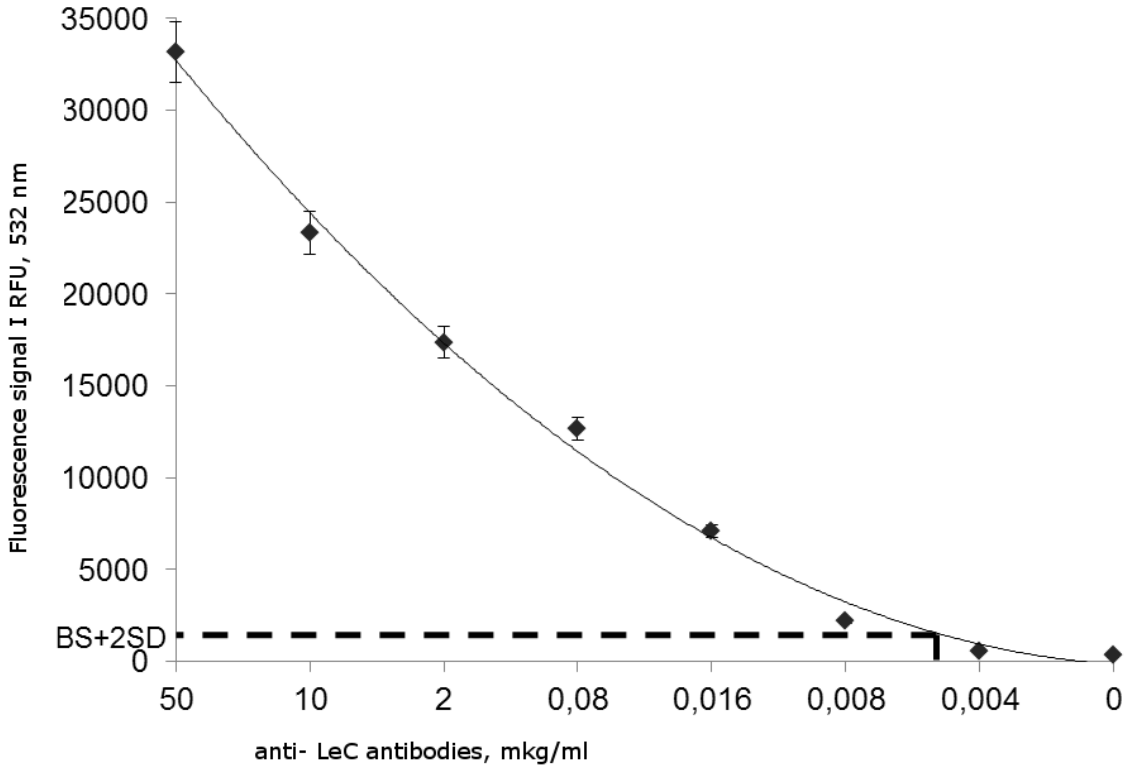


Fig. 1S. Supplementary. Dependence of the fluorescent signal obtained from cells with immobilized LeC from the concentration of antibodies against LeC (1.7 pmol glycan per gel element). The glycochip was incubated with anti-LeC antibodies, then treated with a mixture of a Cy5-labeled anti-IgG and Cy3-labeled anti-IgM (0.005 mg/mL) antibodies. Each point on the calibration curve is the average of two experiments. The minimum detectable concentration of antibodies to LeC was determined as the concentration which fluorescent signal *I* was exceeding the value of the background signal (*BS*) by 2SD (see the section "Fluorescence measurements and analysis results processing").

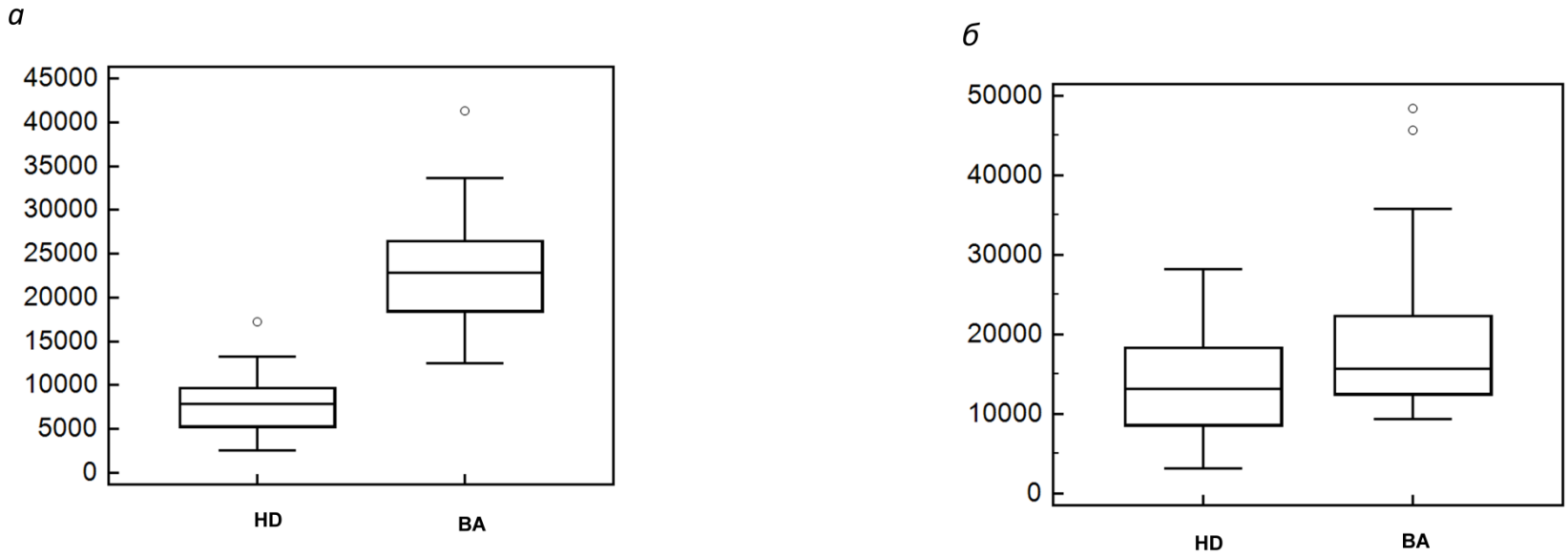


Fig. 2S. Supplementary. Diagrams obtained in the Mann-Whitney test. (a) level of immunoglobulins G and (b) M in cohorts of patients with bronchial asthma (BA) and healthy donors (HD). The borders of the box are the 25th and 75th percentiles, respectively, the line in the middle of the box is the median (the 50th percentile). The ends of the whiskers are the edges of a statistically significant sample (no emissions).

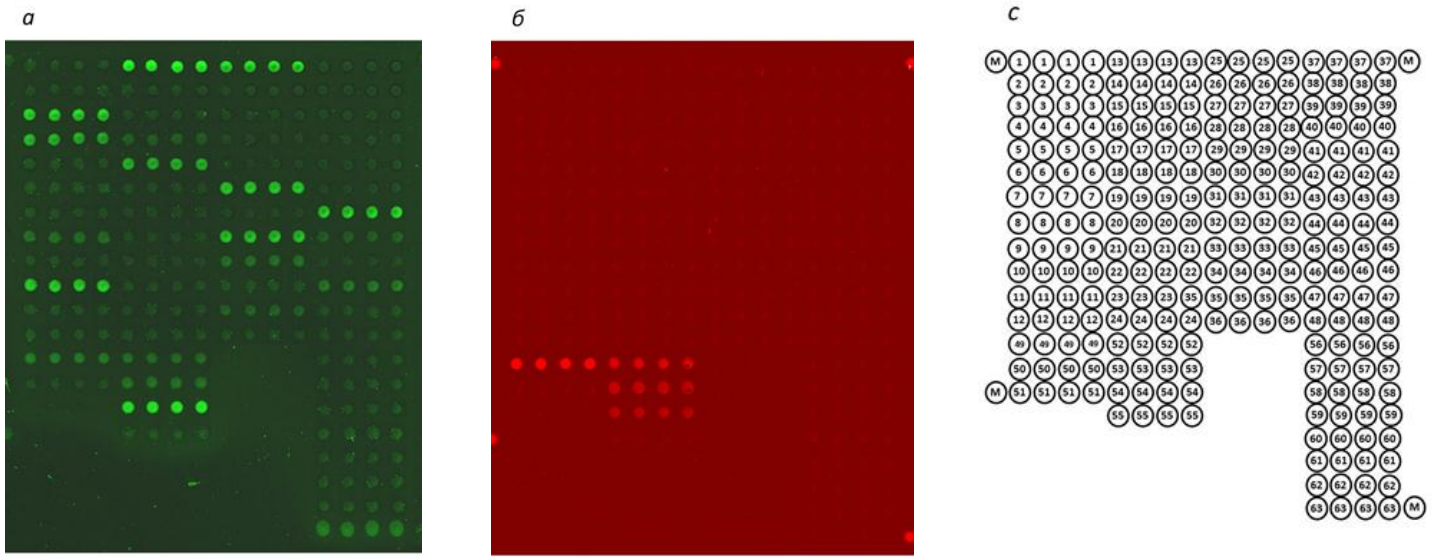


Fig. 3S. Supplementary. Immunofluorescent analysis of a patient's serum on a glycochip. *a* – fluorescent image of the glycochip after incubation with the serum of the patient with bronchial asthma (BA) and developing glycochip with anti-IgM Cy3 (532nm, Cy3); *b* - fluorescent image of the glycochip after incubation with the serum of a healthy donor (HD) and developing glycochip with anti-IgG Cy5 (635nm); *c* - scheme of glycochip (numbers in the circles indicate the positions of the glycan in Table 1S of the Supplementary).

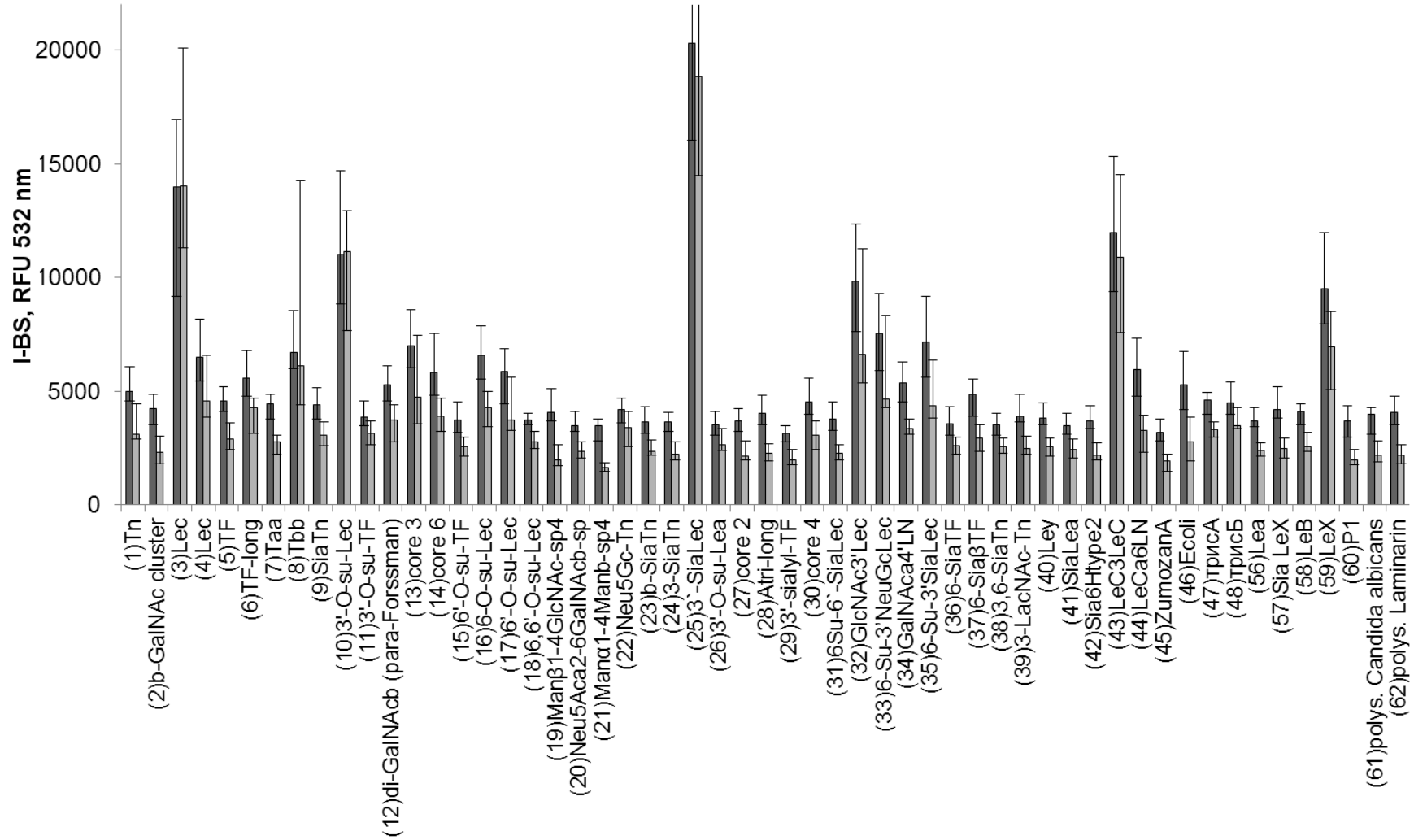
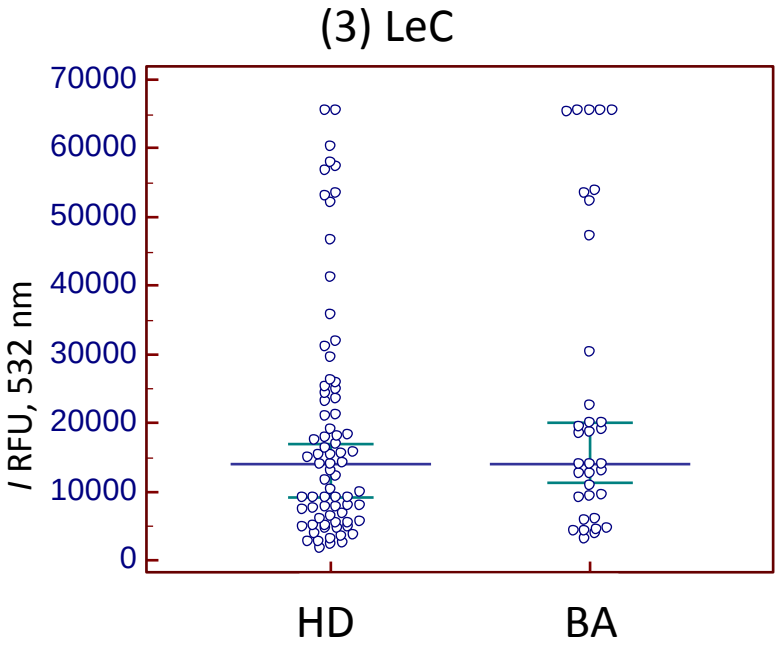
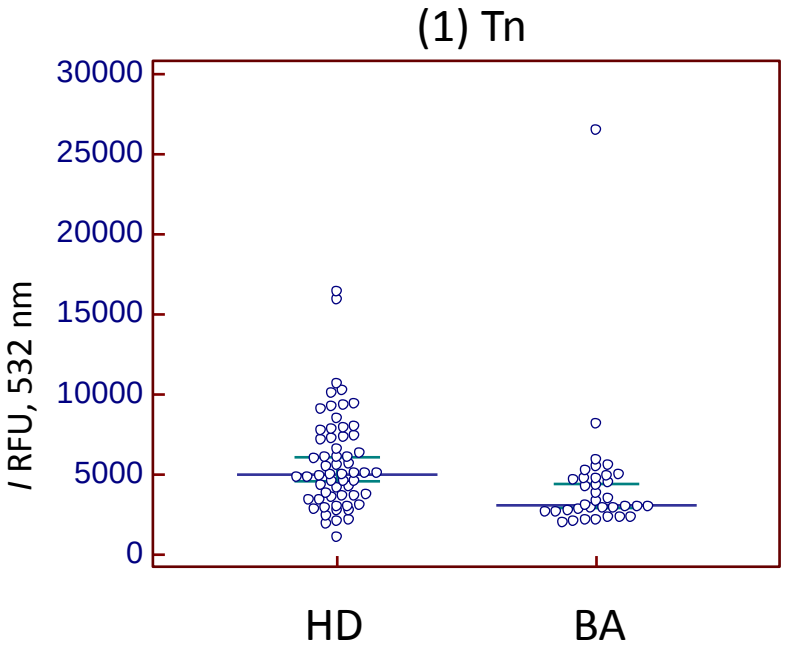


Fig. 4S. Supplementary. Comparison of the fluorescent signals values *I-BS* (for anti-IgM Cy3, 532 nm) obtained from immobilized glycans in the sera of healthy donors (HD) and patients with bronchial asthma (BA). For each glycan 95% confidence interval is indicated.





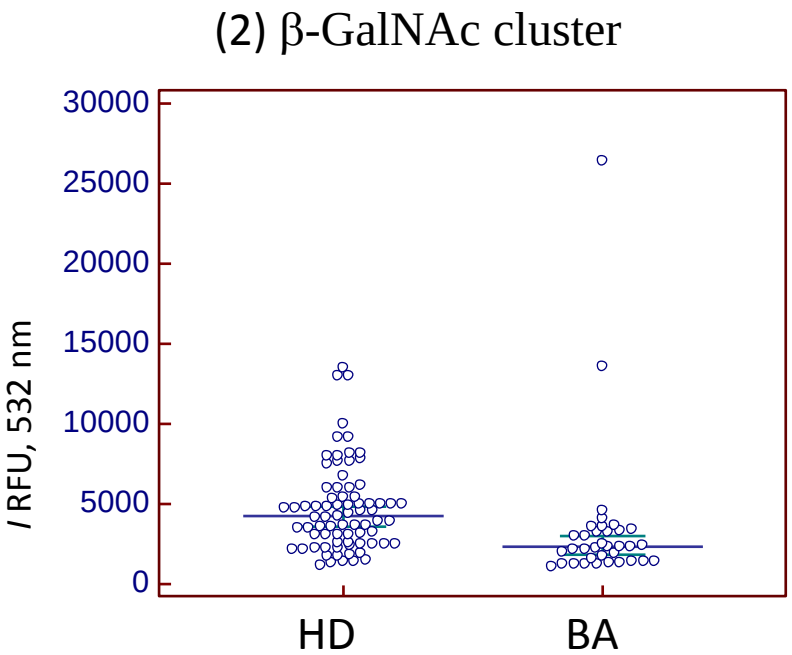
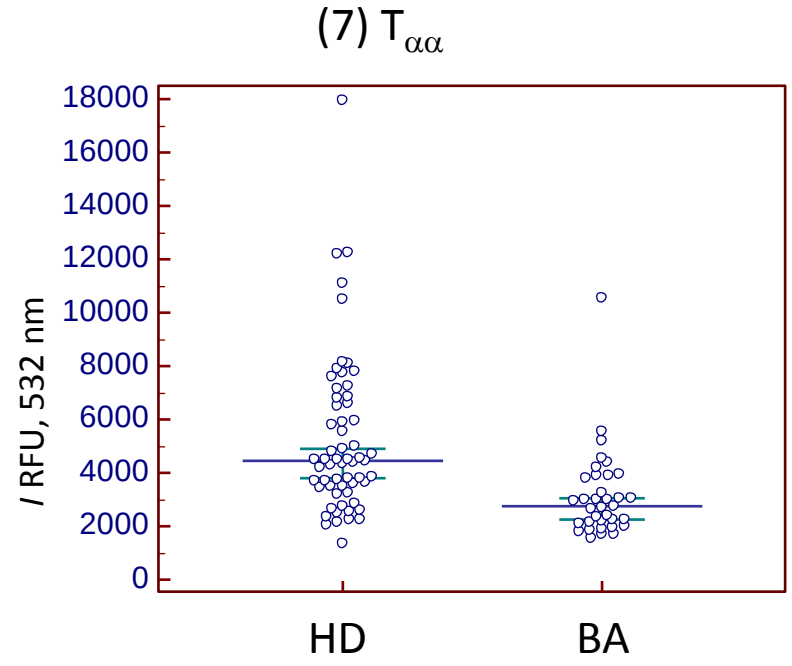
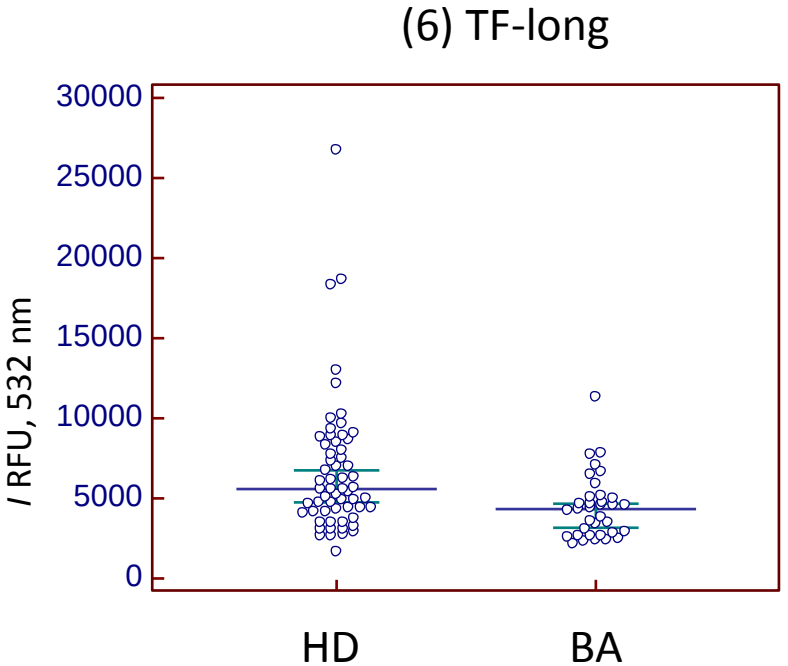
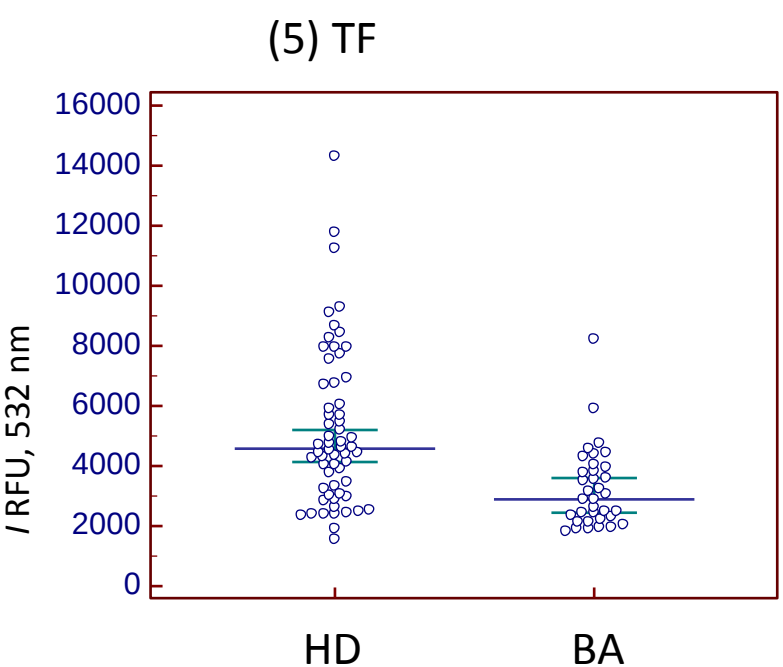
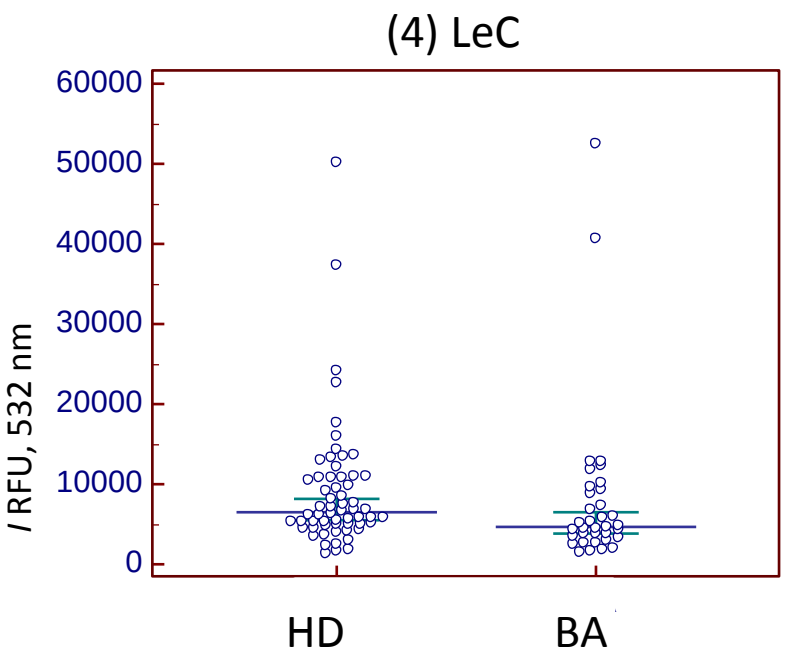


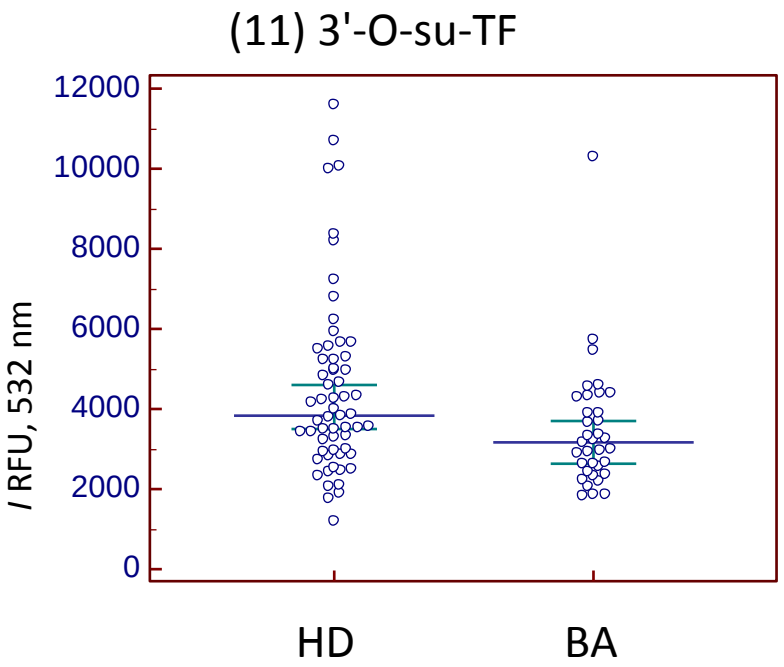
Fig. 5S. Supplementary. Comparison of the fluorescent signals I (for anti-IgM, 532 nm) obtained from immobilized glycans in the sera of healthy donors (HD) and patients with bronchial asthma (BA). For each glycan 95% confidence interval is indicated.

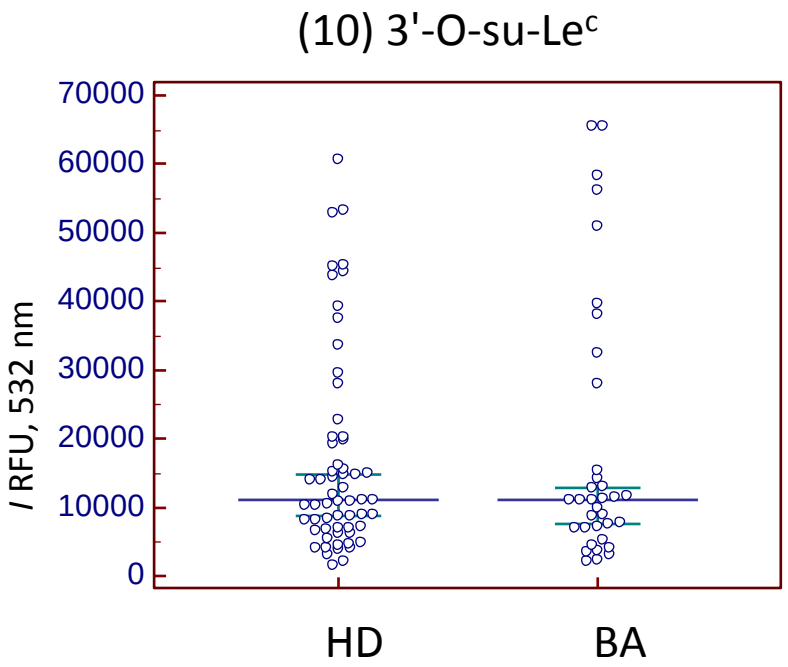

(7) $T_{\alpha\alpha}$
I RFU, 532 nm
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

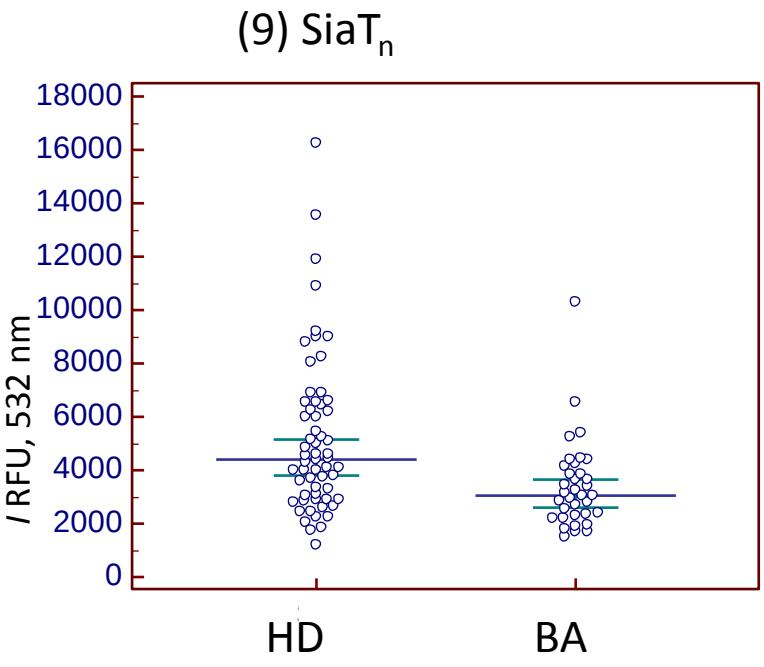

(6) TF-long
I RFU, 532 nm
30000
25000
20000
15000
10000
5000
0
HD
BA

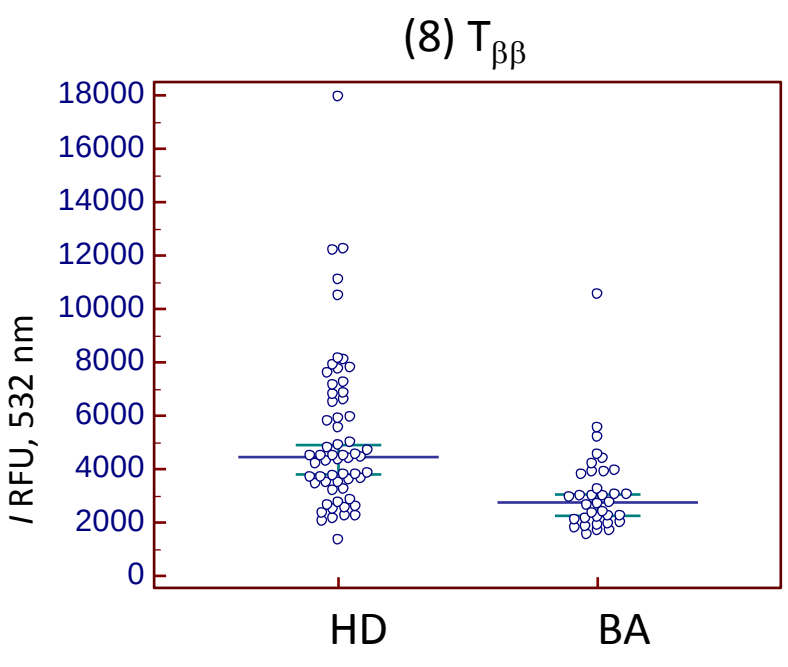

(5) TF
I RFU, 532 nm
16000
14000
12000
10000
8000
6000
4000
2000
0
HD
BA


(4) LeC
I RFU, 532 nm
60000
50000
40000
30000
20000
10000
0
HD
BA

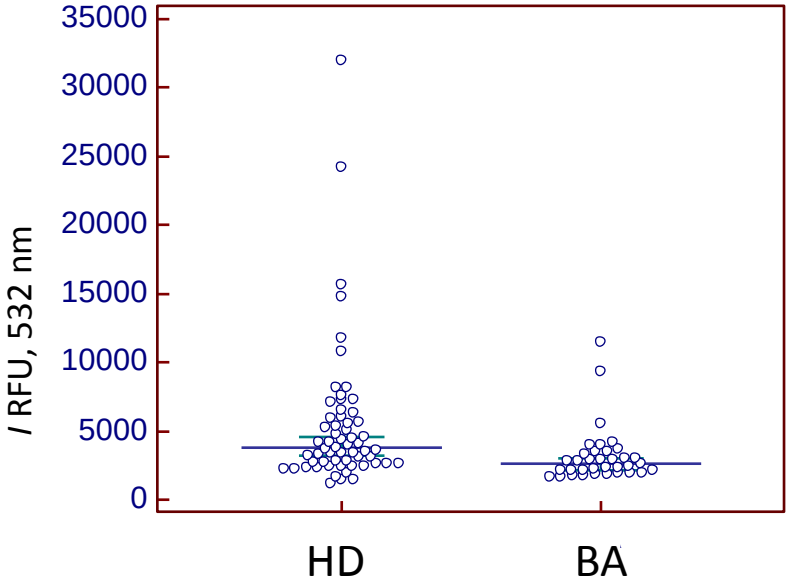

(11) 3'-O-su-TF
I RFU, 532 nm
12000
10000
8000
6000
4000
2000
0
HD
BA


(10) 3'-O-su-Le^c
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA

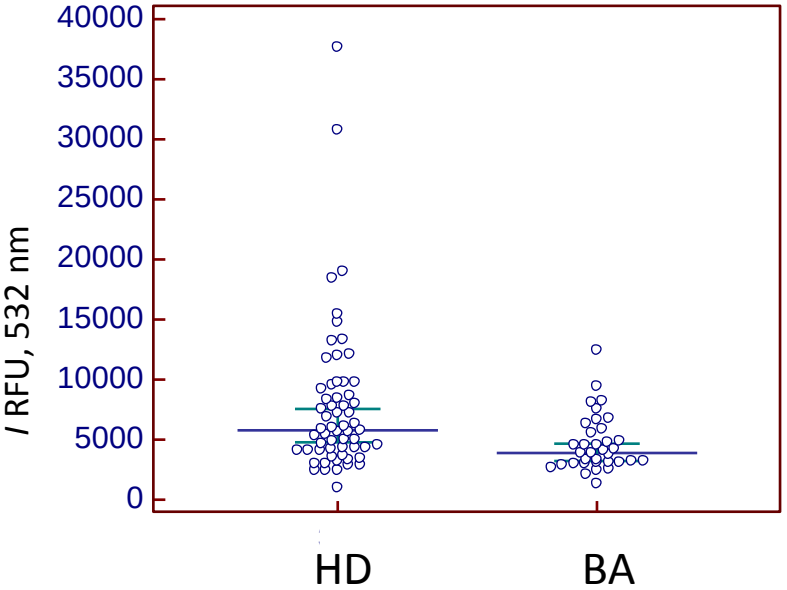

(9) SiaT$_n$
I RFU, 532 nm
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
HD
BA


(8) T$_{\beta\beta}$
I RFU, 532 nm
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

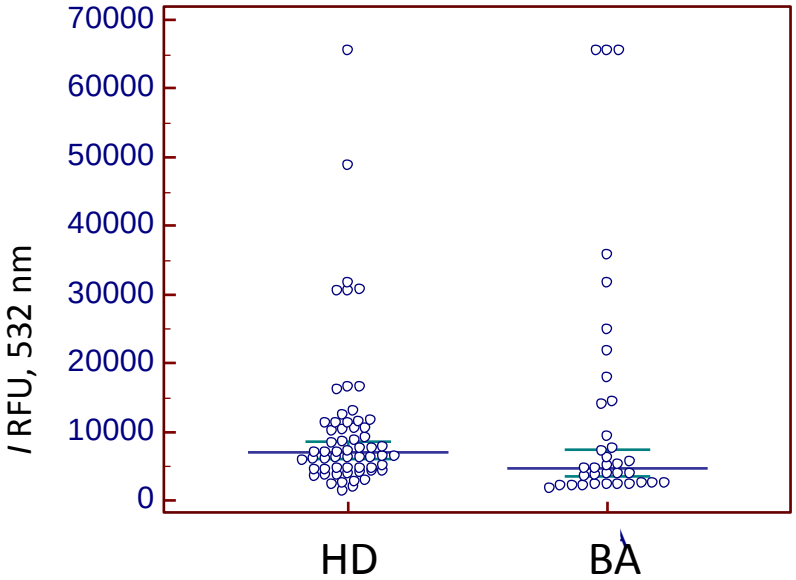
(15) 6'-O-su-TF



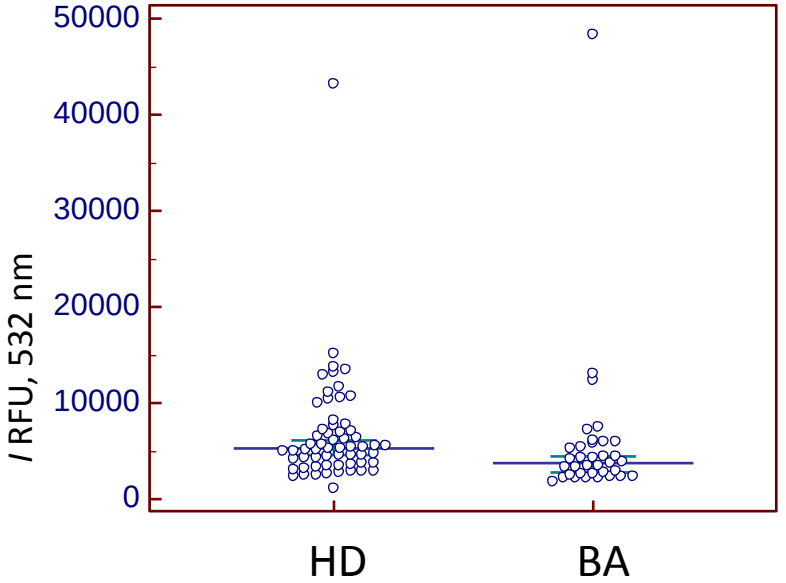
(14) core 6

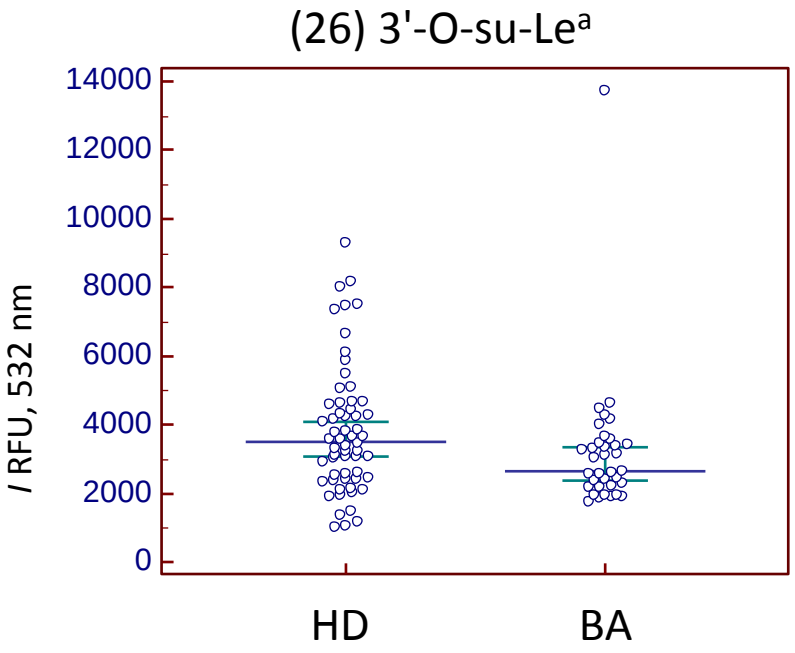


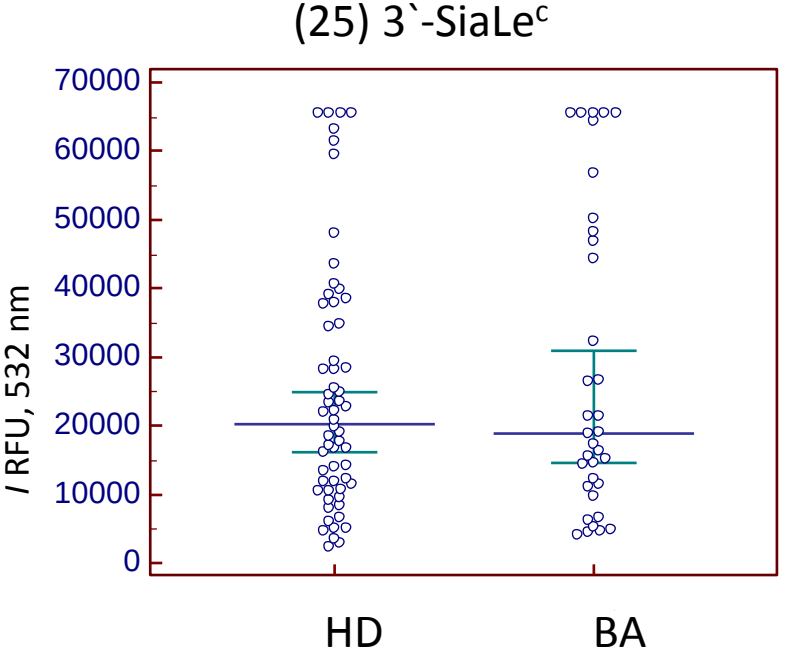
(13) core 3

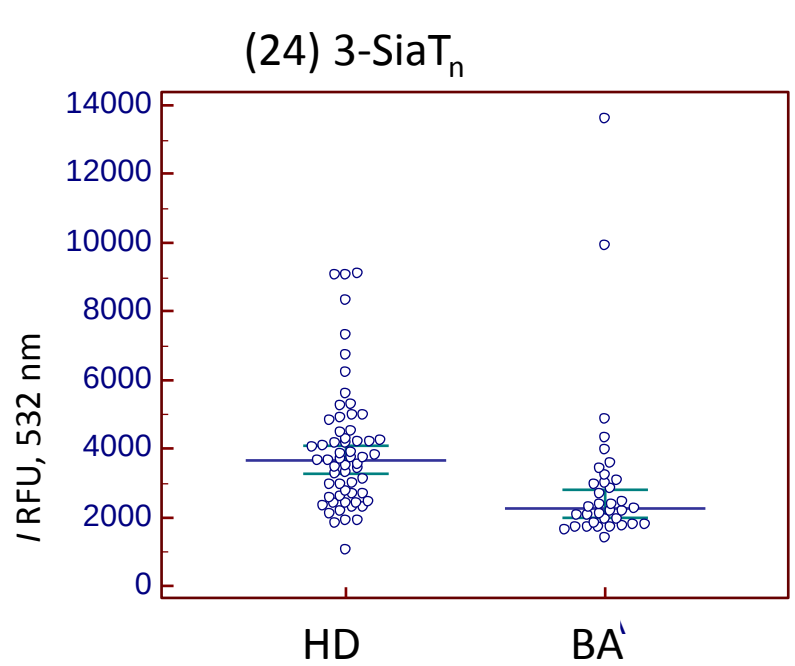


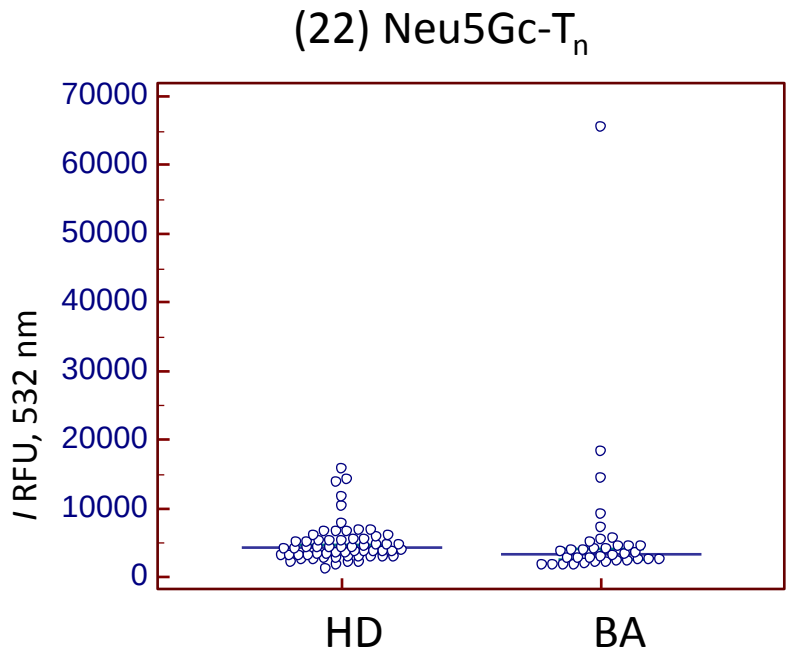
(12) di-GalNAcβ (para-Forssman)



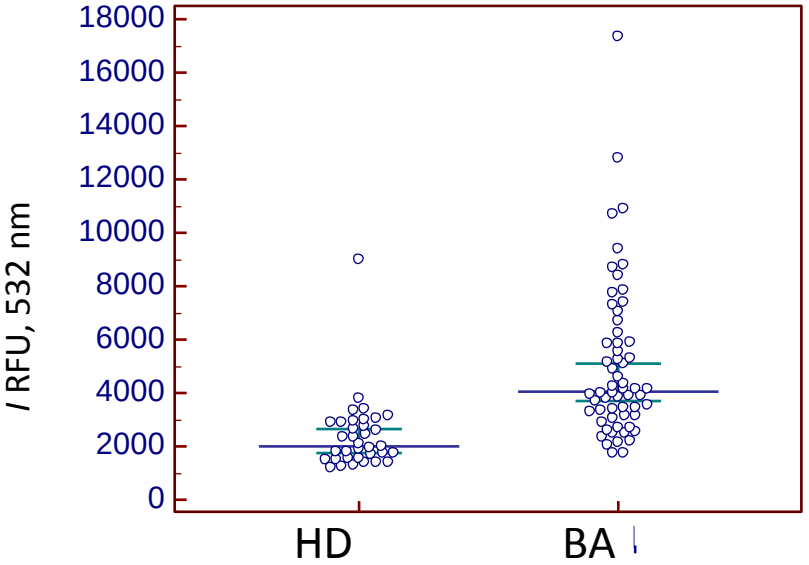

(26) 3'-O-su-Le[a]
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA


(25) 3`-SiaLe[c]
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA

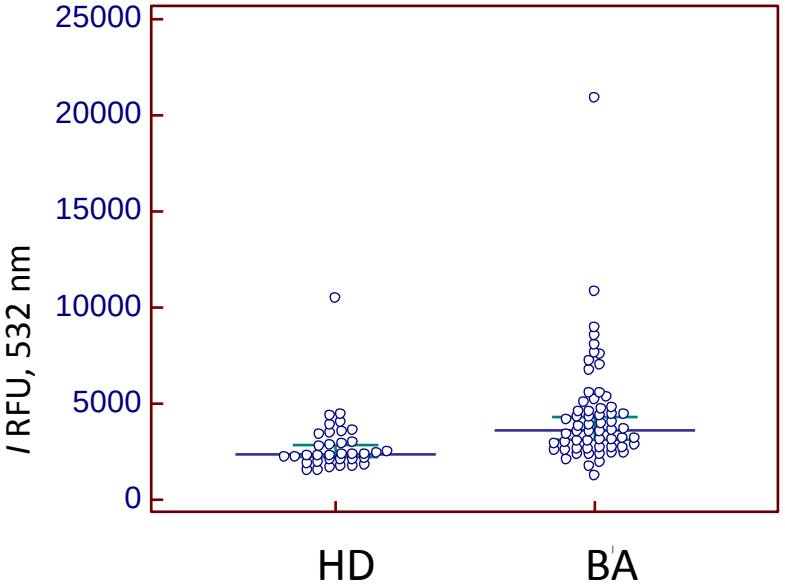

(24) 3-SiaT$_n$
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA


(22) Neu5Gc-T$_n$
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA

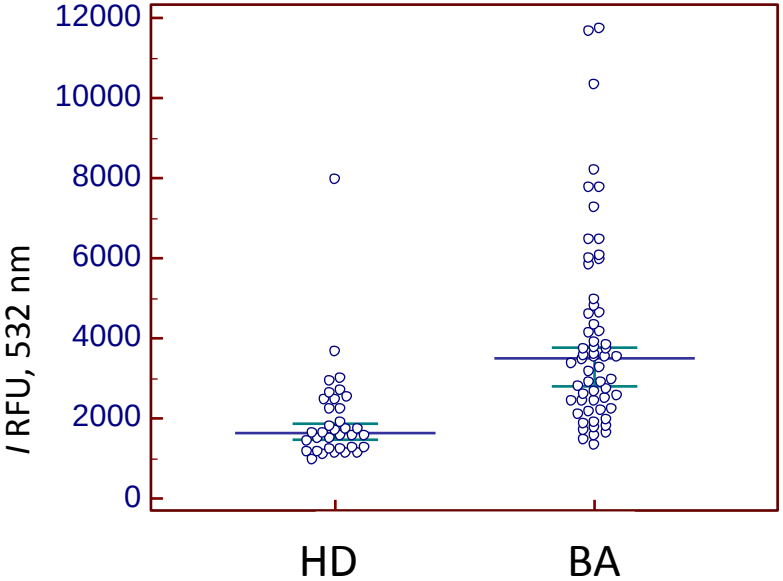
(19) Manβ1-4GlcNAc



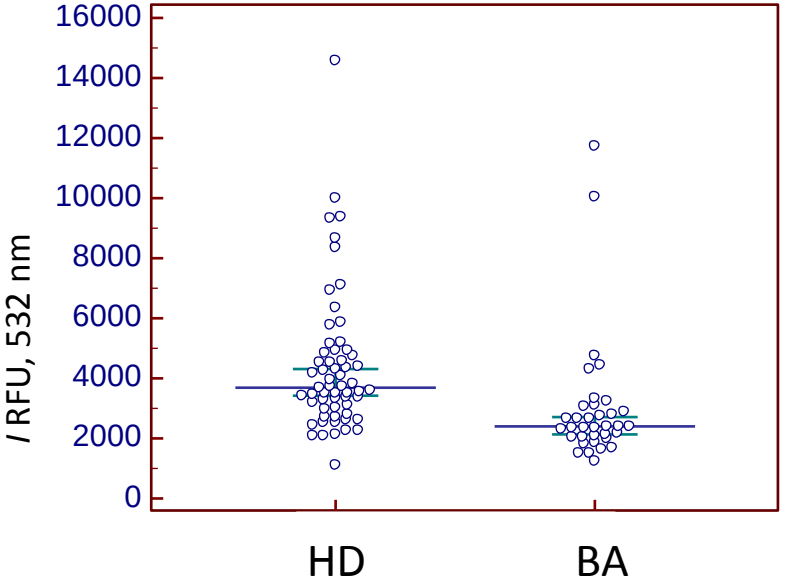
(23) β-SiaT$_n$



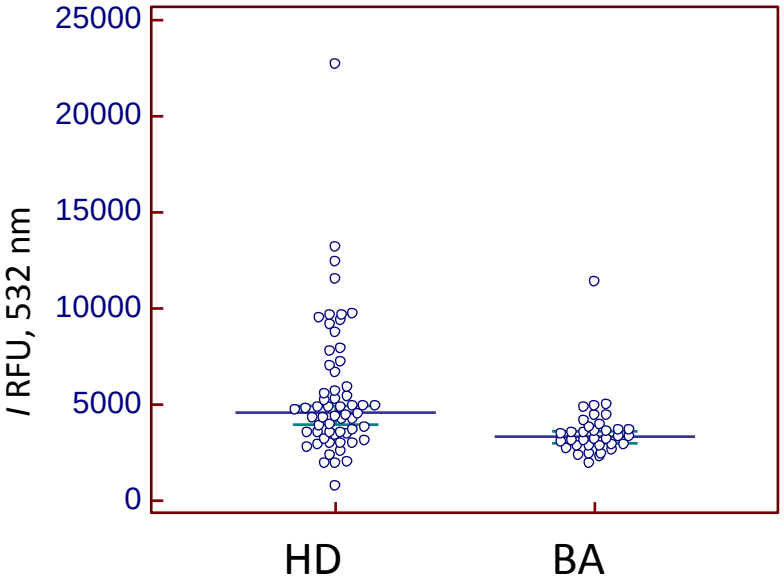
(21) Manα1-4Manβ



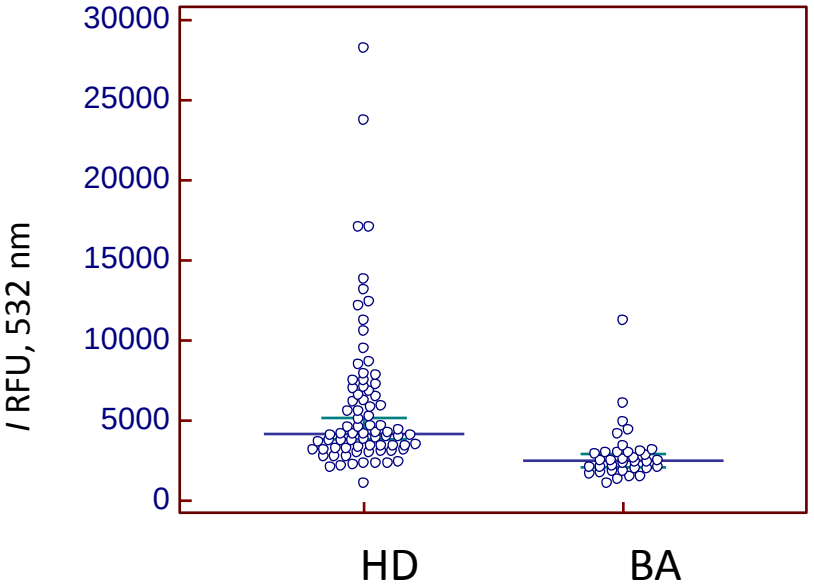
(56) LeA



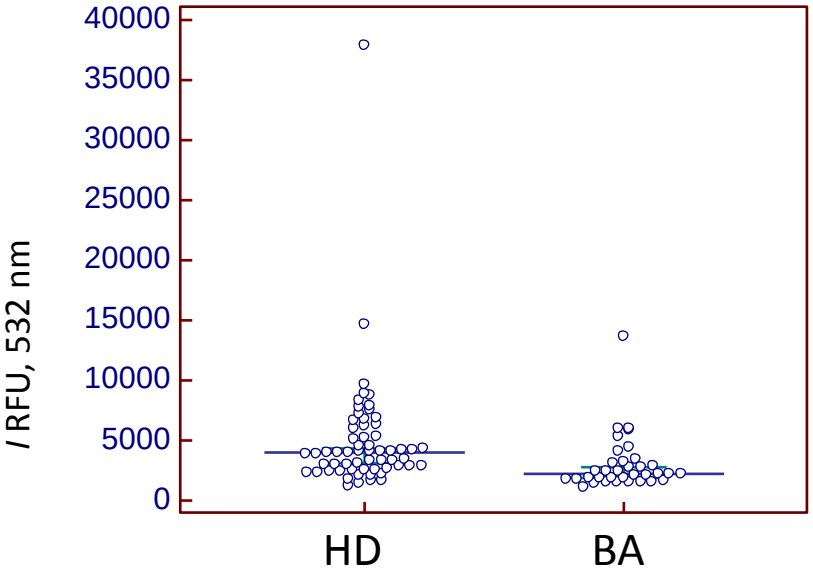
(47) A tri



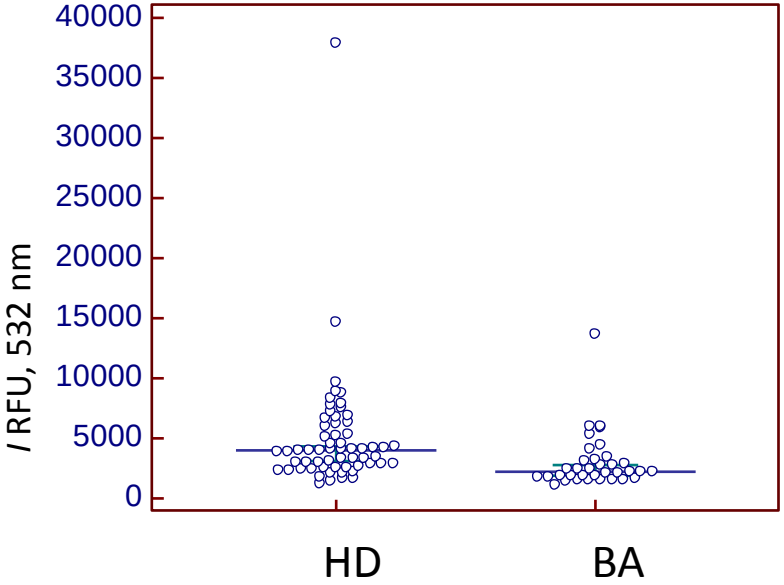
(57) Sia LeX

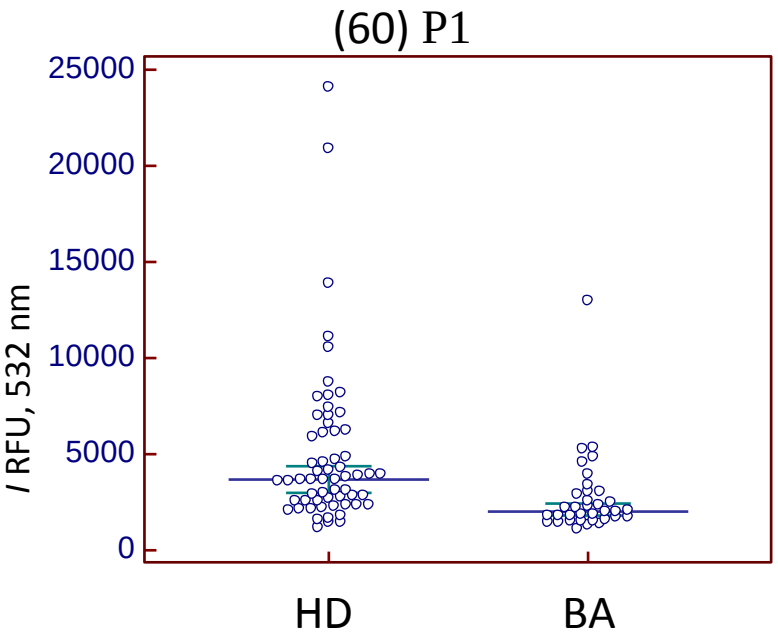


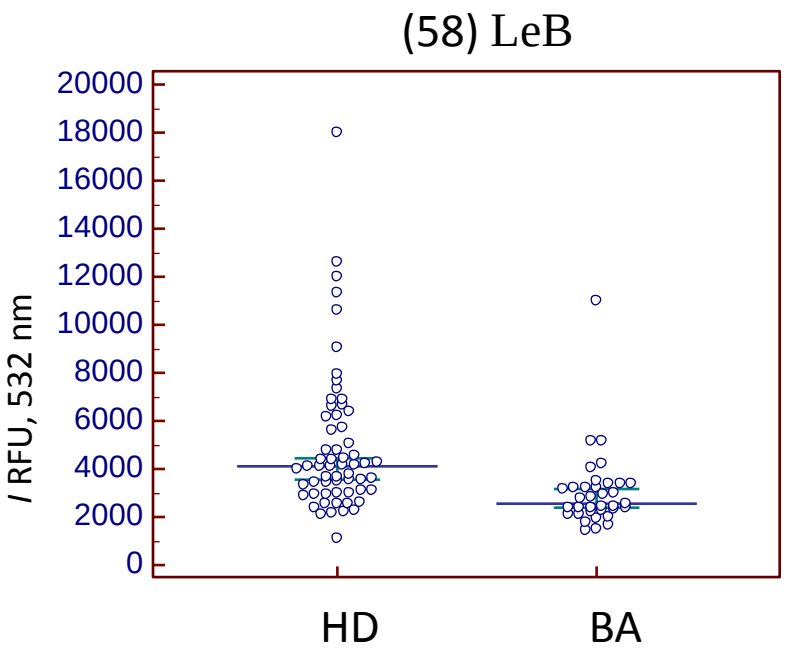
(61) polys. *Candida albicans*

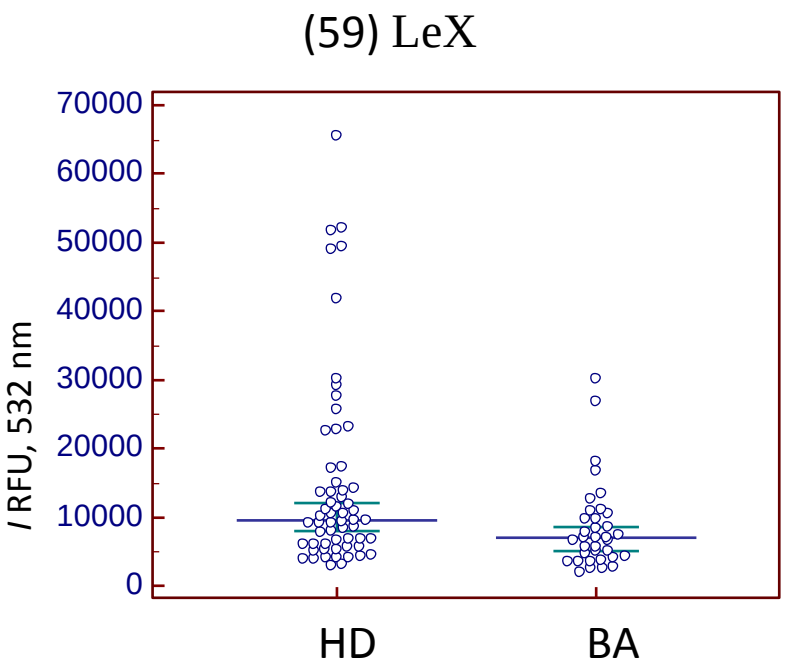


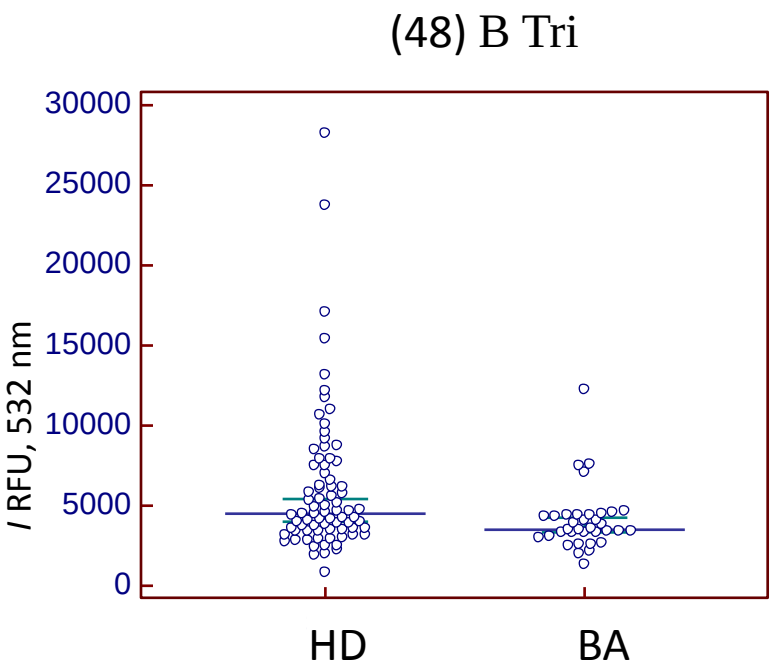
(62) polys. *Laminarin*

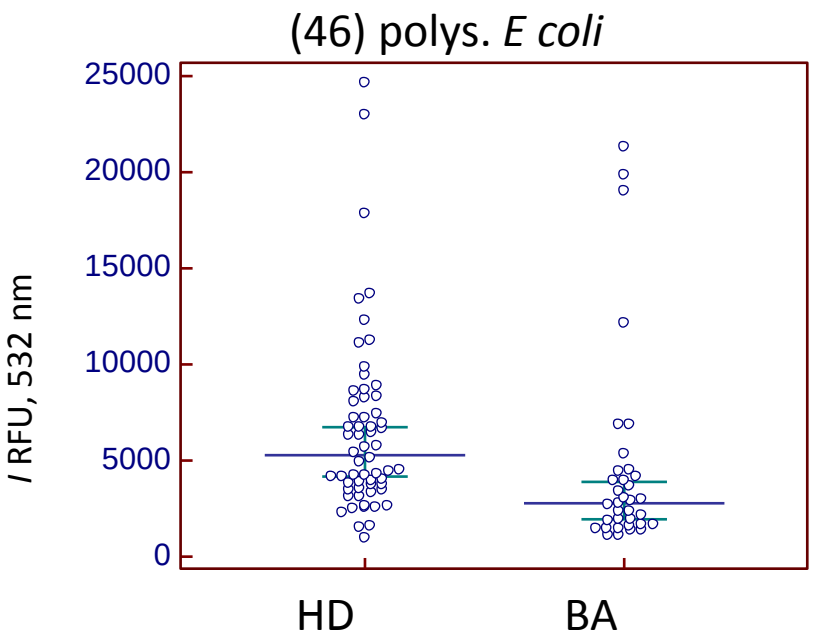


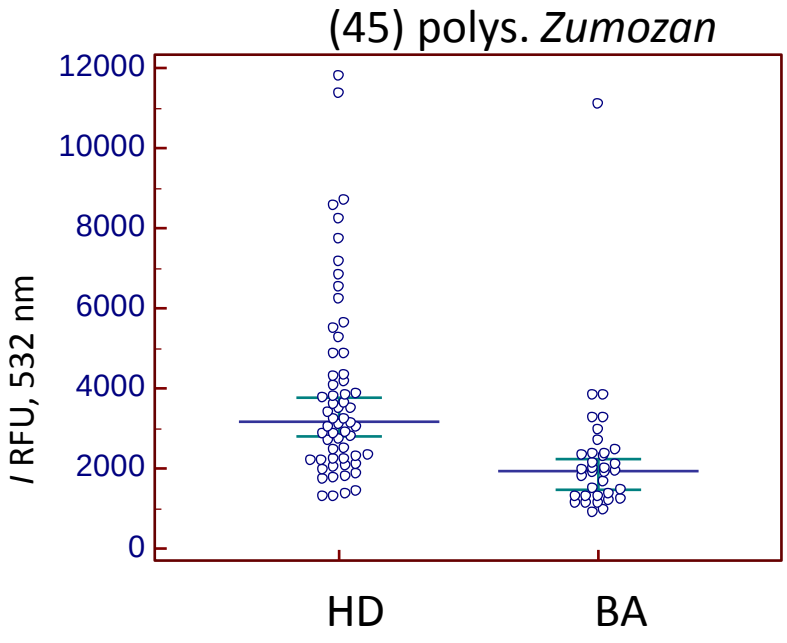

(60) P1
25000
20000
15000
10000
5000
0
I RFU, 532 nm
HD
BA

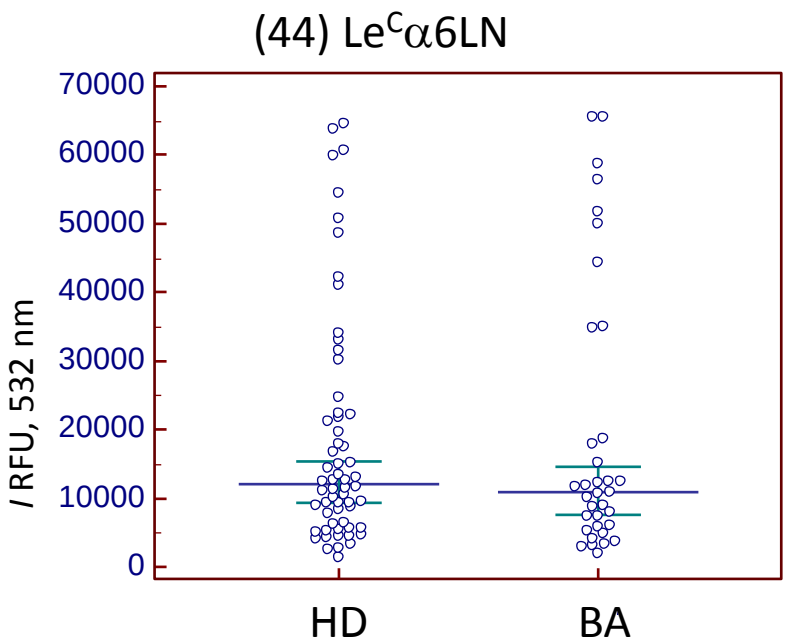

(58) LeB
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
I RFU, 532 nm
HD
BA

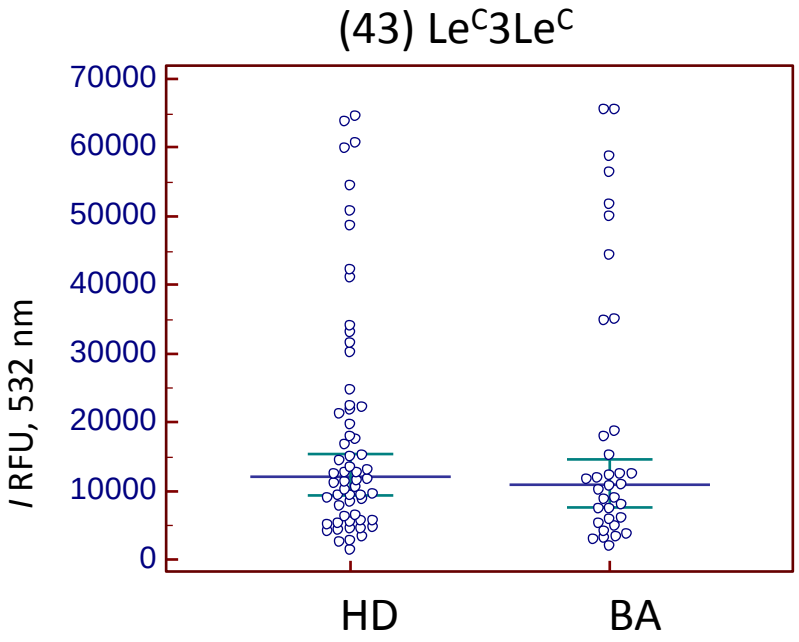

(59) LeX
70000
60000
50000
40000
30000
20000
10000
0
I RFU, 532 nm
HD
BA

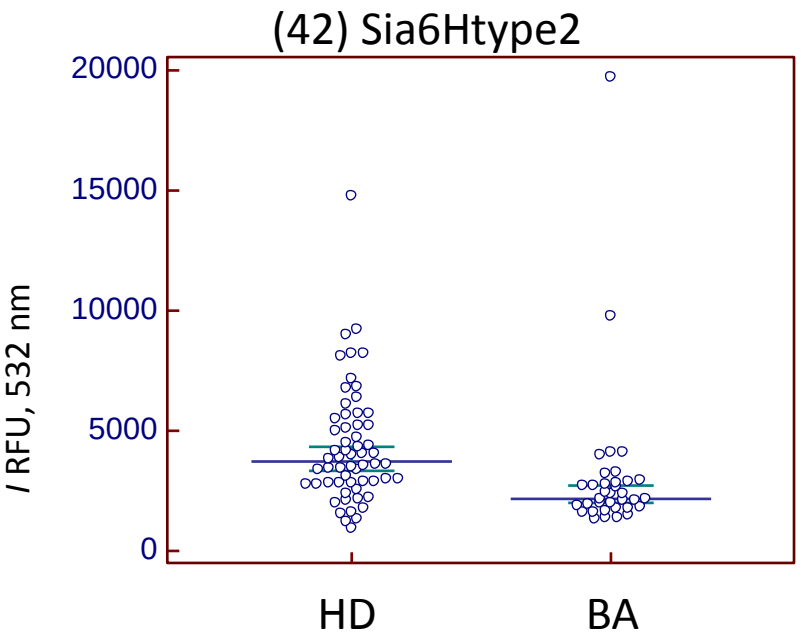

(48) B Tri
30000
25000
20000
15000
10000
5000
0
I RFU, 532 nm
HD
BA

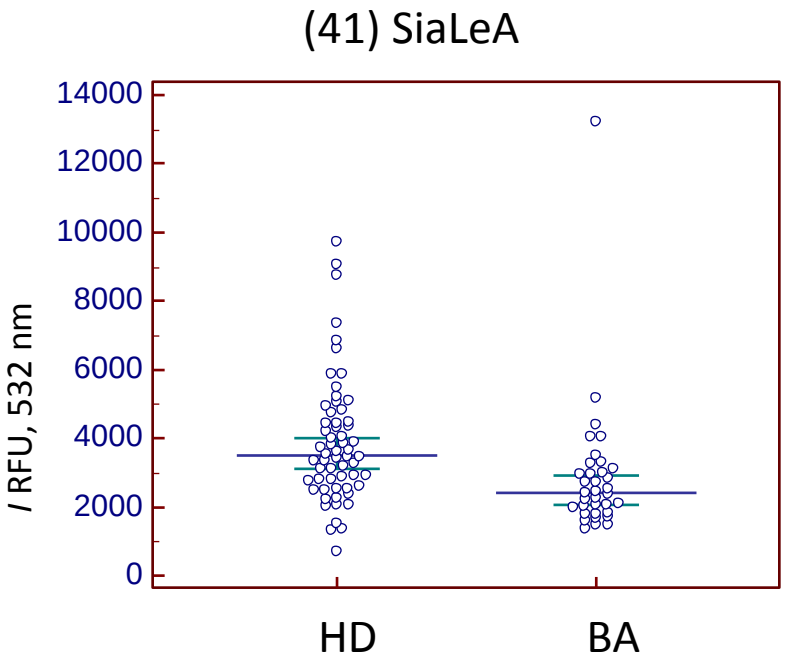

(46) polys. *E coli*
I RFU, 532 nm
25000
20000
15000
10000
5000
0
HD
BA

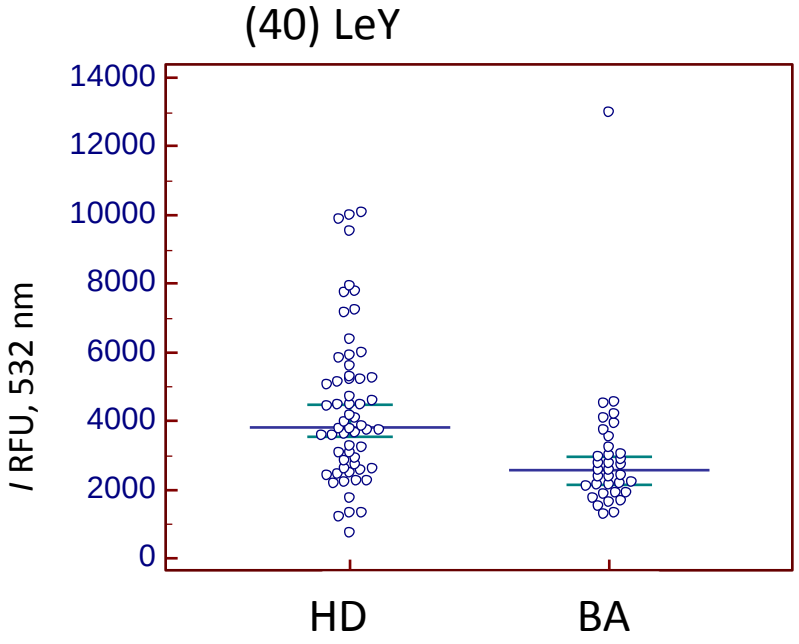

(45) polys. *Zumozan*
I RFU, 532 nm
12000
10000
8000
6000
4000
2000
0
HD
BA

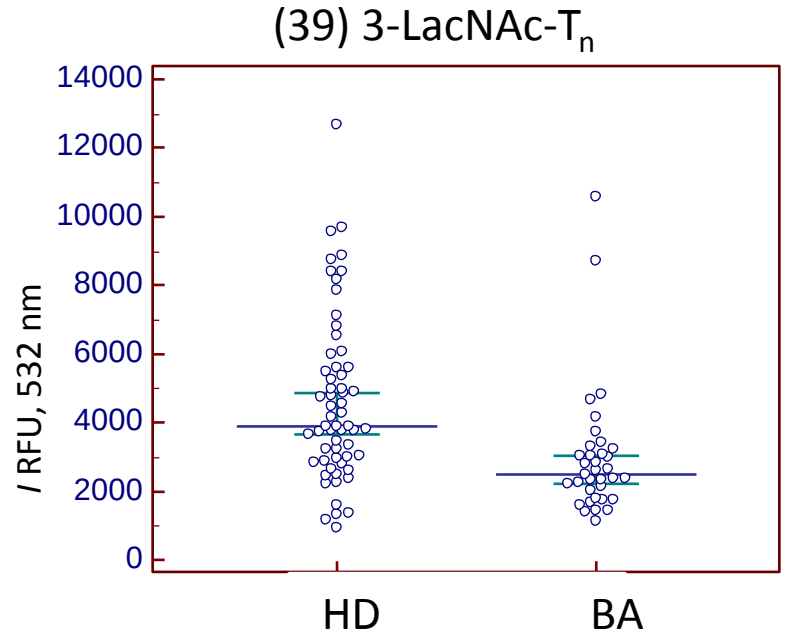

(44) Le^{C}α6LN
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA

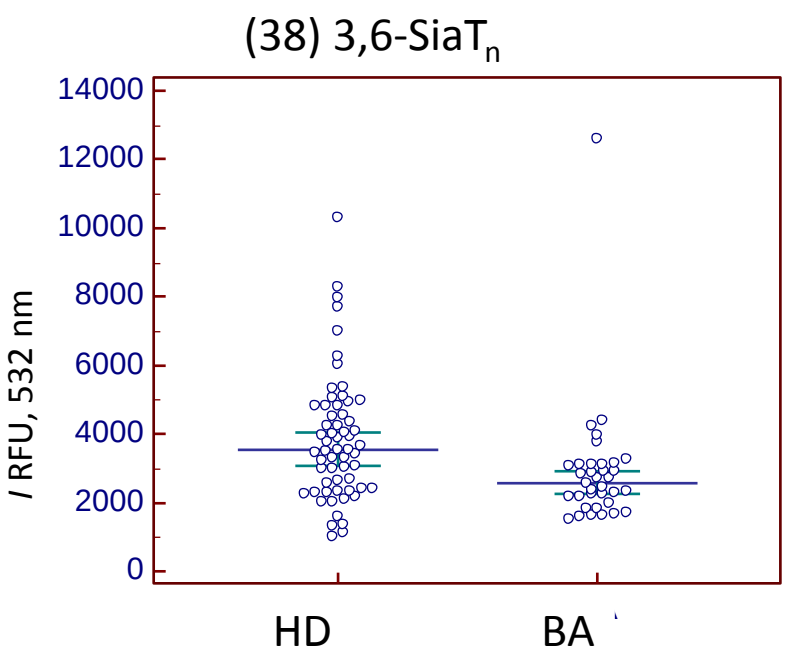

(43) Le^{C}3Le^C
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA

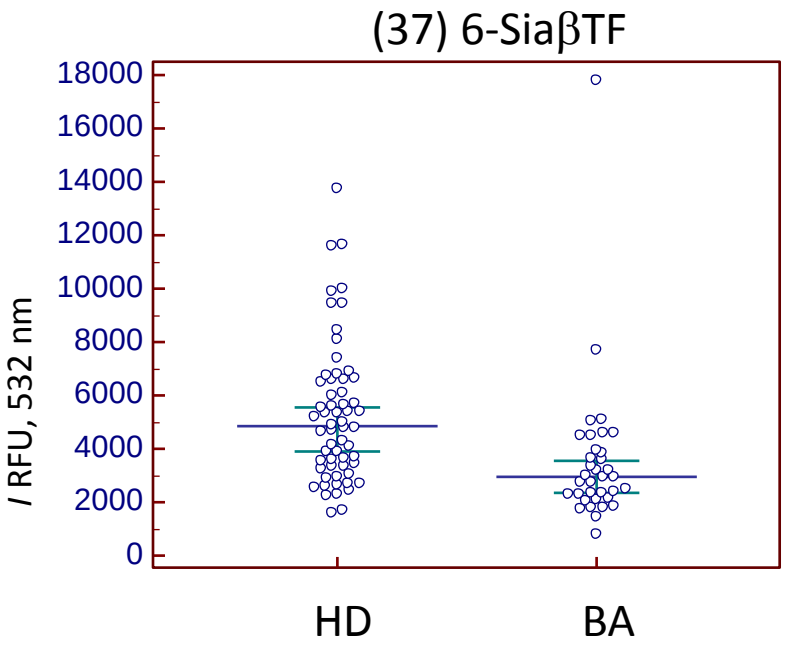

(42) Sia6Htype2
I RFU, 532 nm
20000
15000
10000
5000
0
HD
BA

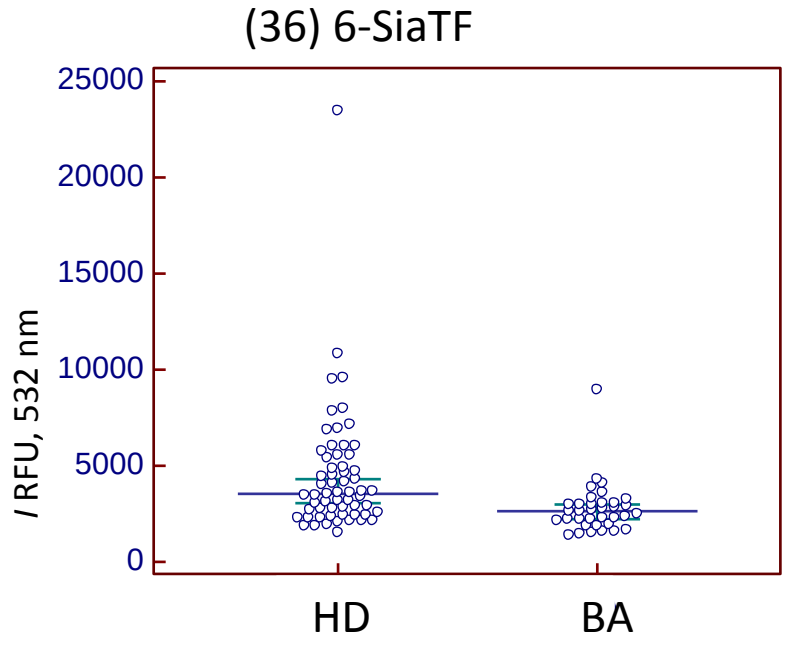

(41) SiaLeA
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

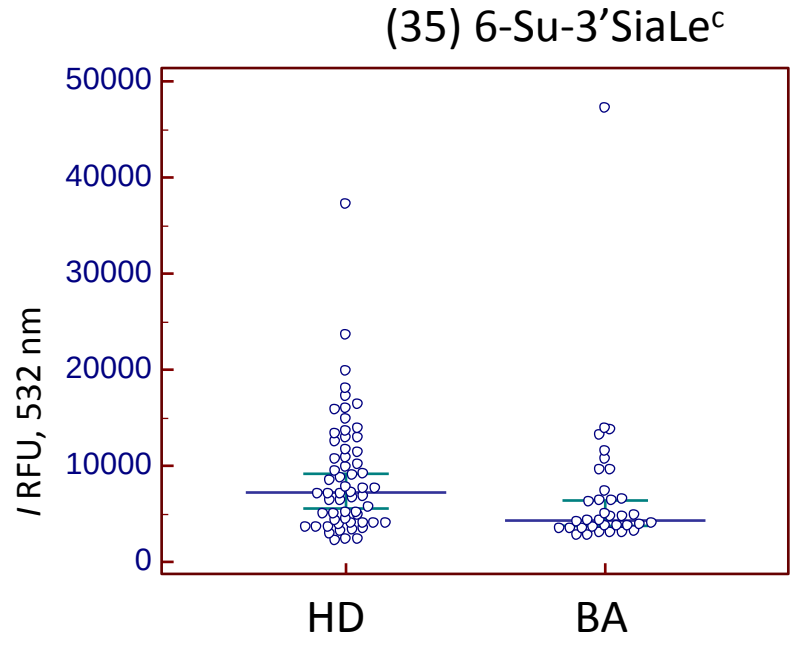

(40) LeY
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

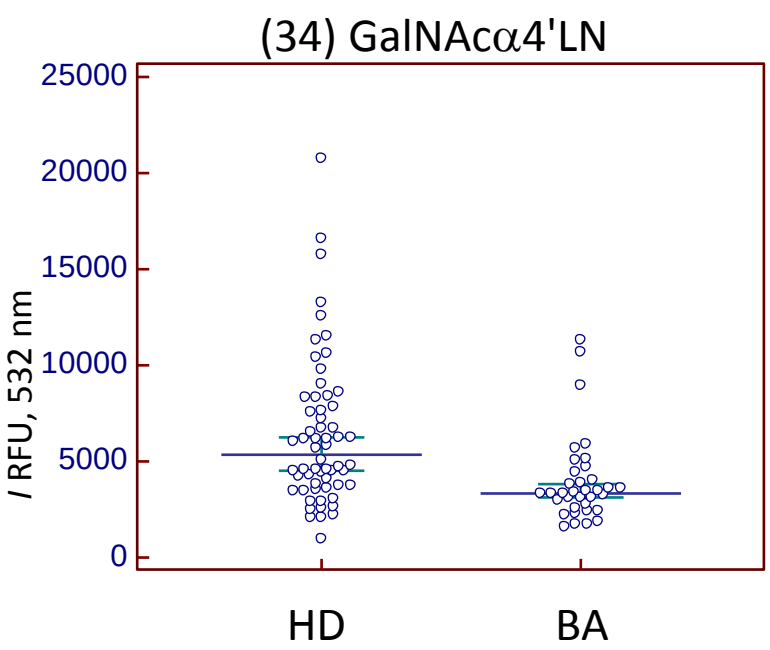

(39) 3-LacNAc-Tn
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

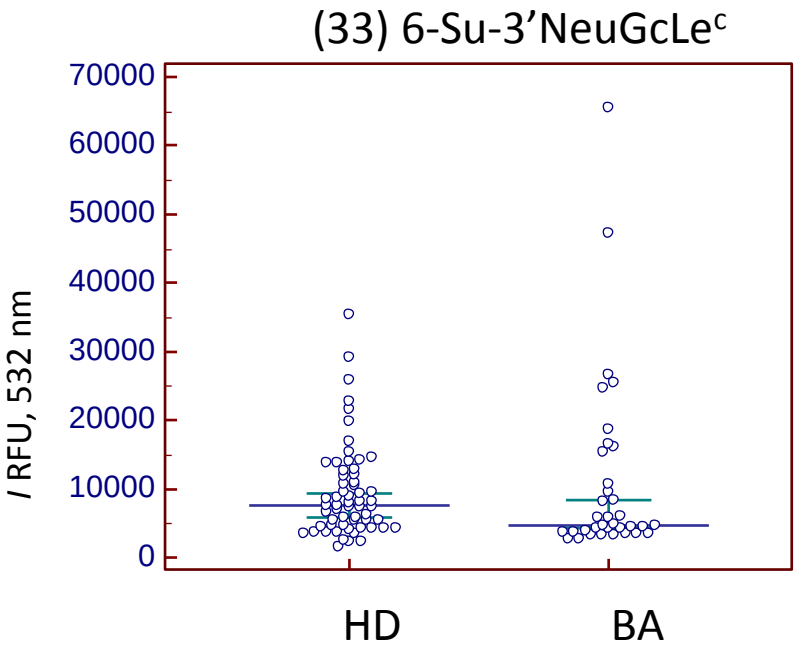

(38) 3,6-SiaTn
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

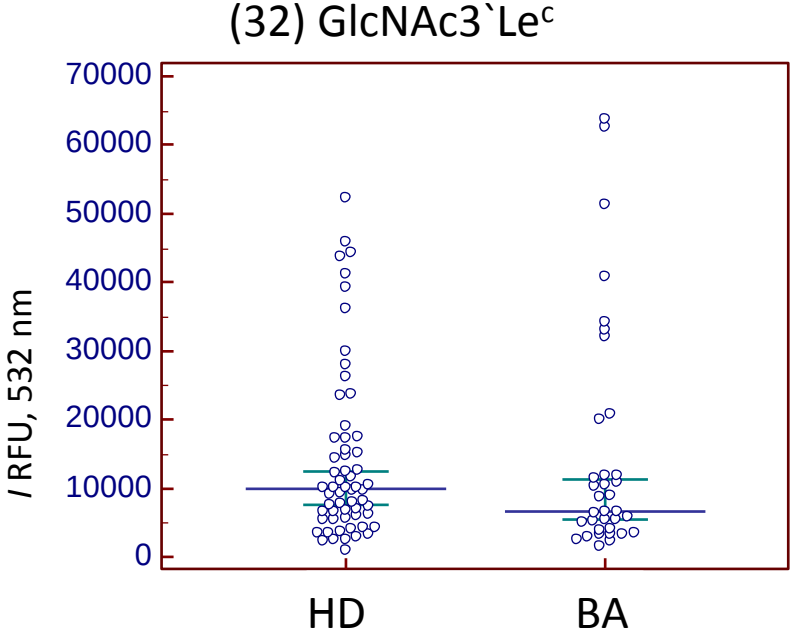

(37) 6-SiaβTF
I RFU, 532 nm
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
HD
BA

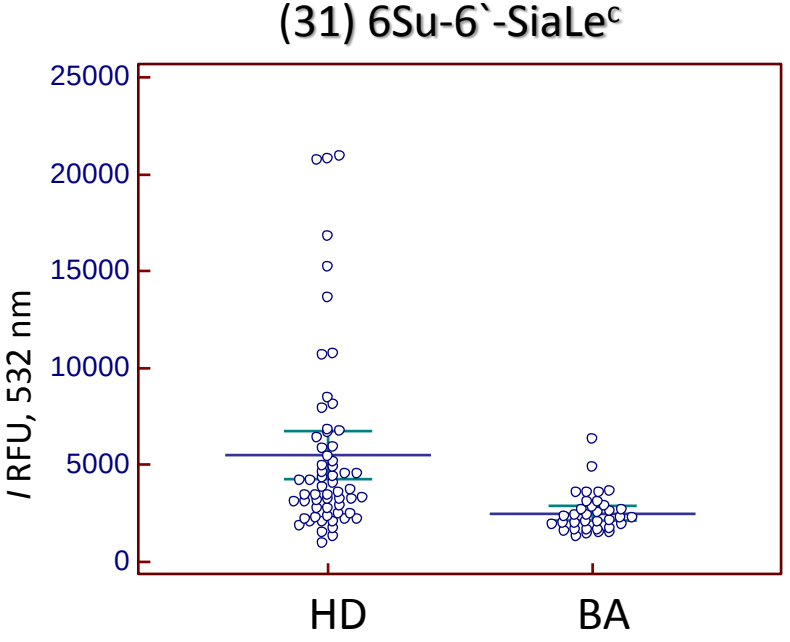

(36) 6-SiaTF
I RFU, 532 nm
25000
20000
15000
10000
5000
0
HD
BA

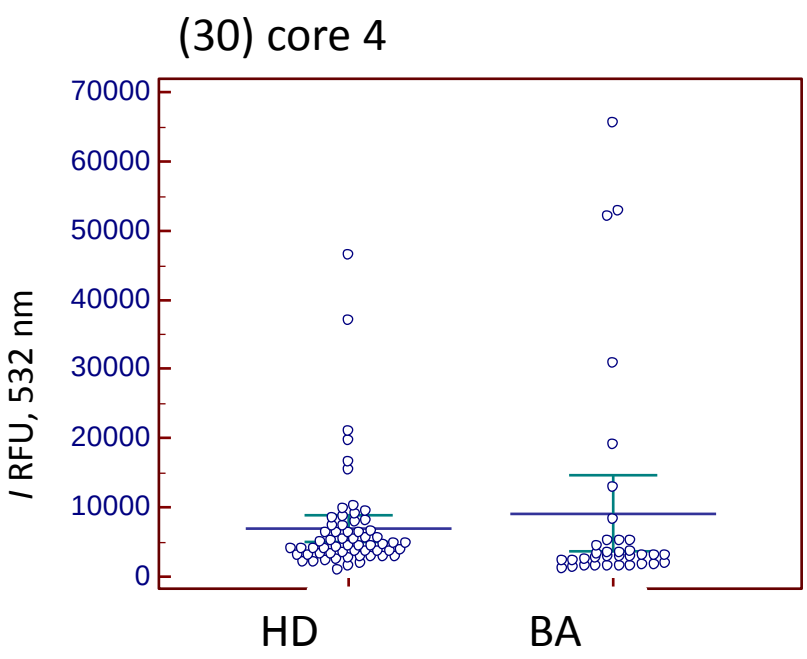

(35) 6-Su-3'SiaLec
I RFU, 532 nm
50000
40000
30000
20000
10000
0
HD
BA

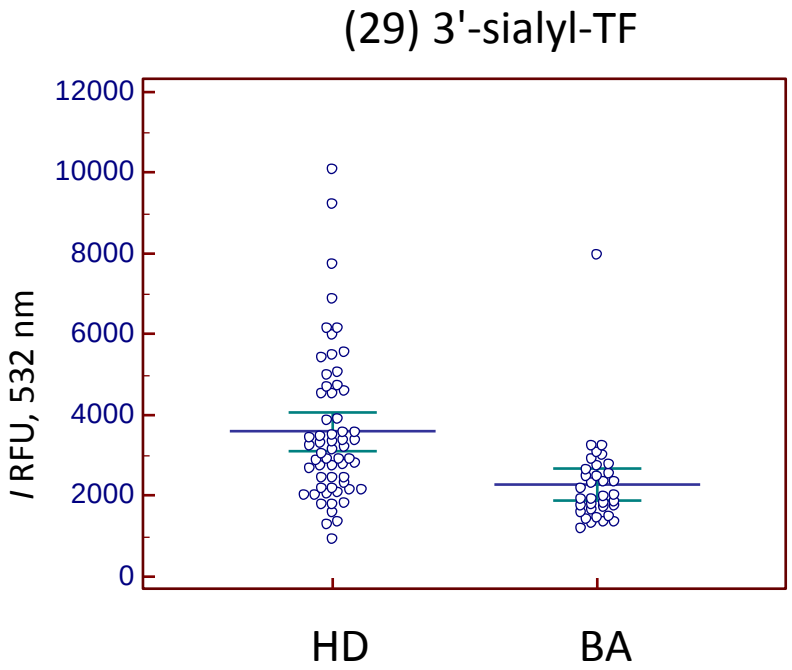

(34) GalNAcα4'LN
I RFU, 532 nm
25000
20000
15000
10000
5000
0
HD
BA

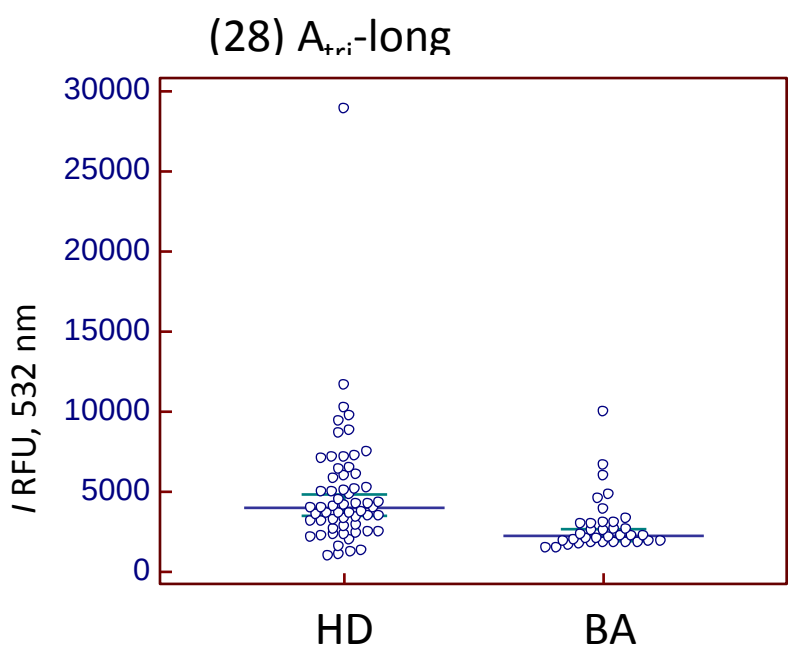

(33) 6-Su-3'NeuGcLe^c
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA

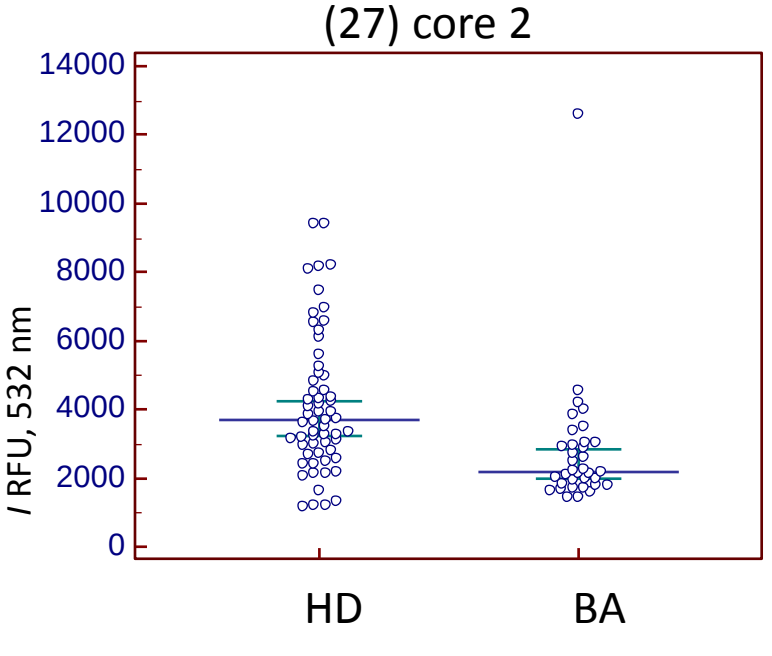

(32) GlcNAc3`Le^c
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA


(31) 6Su-6`-SiaLe^c
I RFU, 532 nm
25000
20000
15000
10000
5000
0
HD
BA


(30) core 4
I RFU, 532 nm
70000
60000
50000
40000
30000
20000
10000
0
HD
BA


(29) 3'-sialyl-TF
I RFU, 532 nm
12000
10000
8000
6000
4000
2000
0
HD
BA


(28) A$_{tri}$-long
I RFU, 532 nm
30000
25000
20000
15000
10000
5000
0
HD
BA


(27) core 2
I RFU, 532 nm
14000
12000
10000
8000
6000
4000
2000
0
HD
BA