Electronic supplementary material (ESM)

Hyaluronan deposition in islets may precede and direct the location of islet immune cell infiltrates

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ESM Table 1. Clinical and morphologic characteristics of study cases

Donor	Donor ID	Autoantibody	Age	Gender	Ethnicity	C-peptide (ng/ml)	BMI	DRB1_1	DRB1_2	DQA1_1	DQA1_2	DQB1_1	DQB1_2	RISK (genotype)	RISK (haplotypes)	Family history for diabetes	Cause of death	Donor group	Islet HA+ area (μm^2)	Beta cell mass (mg)
Autoantibody-positive																				
1	6267	GADA$^+$ IA-2A$^+$	23	Female	Caucasian	16.6	24	0401	0404	0301	0301	0302	0302	S	S / S	nr	Anoxia	HA^{high}LCA^{high}	2450	470
2	6167	IA2A$^+$ ZnT8A$^+$	37	Male	Caucasian	5.4	26	0404	1502	0103	0301	0302	0601	S	S / N	nr	Head trauma	HA^{high}LCA^{high}	1000	1100
3	6158	GADA$^+$ mIAA$^+$	40	Male	Caucasian	0.5	30	0401	1302	0102	0301	0301	0604	P	P / N	Father with T2D	Head trauma	HA^{high}LCA^{high}	900	1160
4	6154	GADA$^+$	49	Female	Caucasian	0.1	25	0901	1501	0102	0301	0303	0603	P	N / P	nr	Head trauma	HA^{high}	800	610
5	6310	GADA$^+$	28	Female	Hispanic	10.5	24	0701	1102	0201	0501	0202	0319	N	N / N	nr	Anoxia	HA^{high}	680	450
6	6197	GADA$^+$ IA-2A$^+$	22	Male	African-American	17.5	28	0302	0701	02:01	0401	0202	0402	P	P / P	Yes, unspecified	Head trauma	HA^{high}	660	1230
7	6151	GADA$^+$	30	Male	Caucasian	5.5	24	0101	0701	0101	0201	0202	0501	N	N / N	nr	Anoxia	HA^{high}	600	240
8	6080	GADA$^+$ mIAA$^+$	69	Female	Caucasian	1.84	21	0101	0401	0101	0301	0301	0501	P	N / P	nr	Cerebrovascular/ stroke	HA^{low}	380	910
9	6027	ZnT8$^+$	19	Male	Caucasian	nd	20	0301	1501	0102	0501	0201	0602	P	S / P	nr	nr	HA^{low}	250	790
10	6171	GADA$^+$	4	Female	Caucasian	9.0	15	0301	0301	0501	0501	0201	0201	S	S / S	nr	Anoxia	HA^{low}	250	N/D
11	6303	GADA$^+$	22	Male	Caucasian	3.0	32	0301	0701	0201	0501	0201	0202	S	S / N	Sister with juvenile T1D, father with T2D	Head trauma	HA^{low}	220	850
12	6181	GADA$^+$	32	Male	Caucasian	0.6	22	0101	0401	0101	0301	0302	0501	S	N / S	nr	Head trauma	HA^{low}	200	690
13	6123	GADA$^+$	23	Female	Caucasian	2.0	18	0801	1101	0401	0501	0301	0402	N	N / P	nr	Head trauma	HA^{low}	200	510
14	6301	GADA$^+$	26	Male	African-American	3.9	32	1101	1304	0102	0501	0319	0602	P	P / P	nr	Head trauma	HA^{low}	140	580
15	6314	GADA$^+$	21	Male	Caucasian	1.5	24	0103	0401	0101	0501	0301	0501	P	N / P	Yes, unspecified	Head trauma	HA^{low}	140	730
Average			30 ± 15 years	Females, 40%		6 ± 6	24 ± 5 kg/m^2													
Autoantibody-negative																				
16	6055	Negative	27	Male	Caucasian	0.6	23	0103	0103	0501	0501	0301	0301	P	P / P	nr	Anoxia		180	1350
17	6104	Negative	41	Male	Caucasian	20.6	21	0701	1301	0101	0201	0201	0501	N	N / N	nr	Anoxia		350	450
18	6179	Negative	20	Female	Caucasian	2.7	21	0301	0404	0301	0501	0201	0302	S	S / S	nr	Head trauma		220	660
19	6233	Negative	14	Male	Caucasian	7.3	22	0101	1301	0101	0103	0501	0603	P	N / P	Mother and sister with T1D	Anoxia		330	1180
20	6129	Negative	43	Female	Caucasian	0.5	23	0301	1501	0102	0501	0201	0602	P	P / P	nr	Anoxia		230	690
21	6013	Negative	65	Male	Caucasian	2.8	24	0102	1301	0101	0103	0501	0603	N	N / P	nr	Cerebrovascular/ stroke		370	N/D
22	6230	Negative	16	Male	Caucasian	5.2	19	0401	1101	0301	0501	0301	0302	S	S / P	nr	Head trauma		190	800
23	6174	Negative	21	Male	Caucasian	3.0	20	0301	0701	0201	0501	0201	0201	S	S / N	nr	Cerebrovascular/ stroke		340	860
24	6232	Negative	14	Female	Caucasian	19.5	21	1501	1501	0102	0102	0602	0602	P	P / P	nr	Head trauma		180	460
25	6295	Negative	47	Female	African-American	10.9	30	0301	1501	0102	0501	0201	0602	P	S / P	nr	Head trauma		220	390
26	6005	Negative	5	Female	Caucasian	nd	nd	0101	1201	0101	0501	0301	0501	N	N / N	nr	Cerebrovascular/ stroke		170	N/D
27	6134	Negative	27	Male	Caucasian	3.6	20	0701	1001	0101	0201	0201	0501	N	N / N	nr	Anoxia		290	910
28	6160	Negative	22	Male	Caucasian	0.4	24	nd								nr	Head trauma		260	1070
29	6098	Negative	18	Male	Caucasian	1.4	23	0301	0801	0401	0501	0201	0402	S	S / N	nr	Head trauma		170	1200
Average			27 ± 16 years	Females, 30%		6 ± 7	22 ± 3 kg/m^2													

nr, not reported
nd, not determined
P, protective; N, neutral; S, susceptible

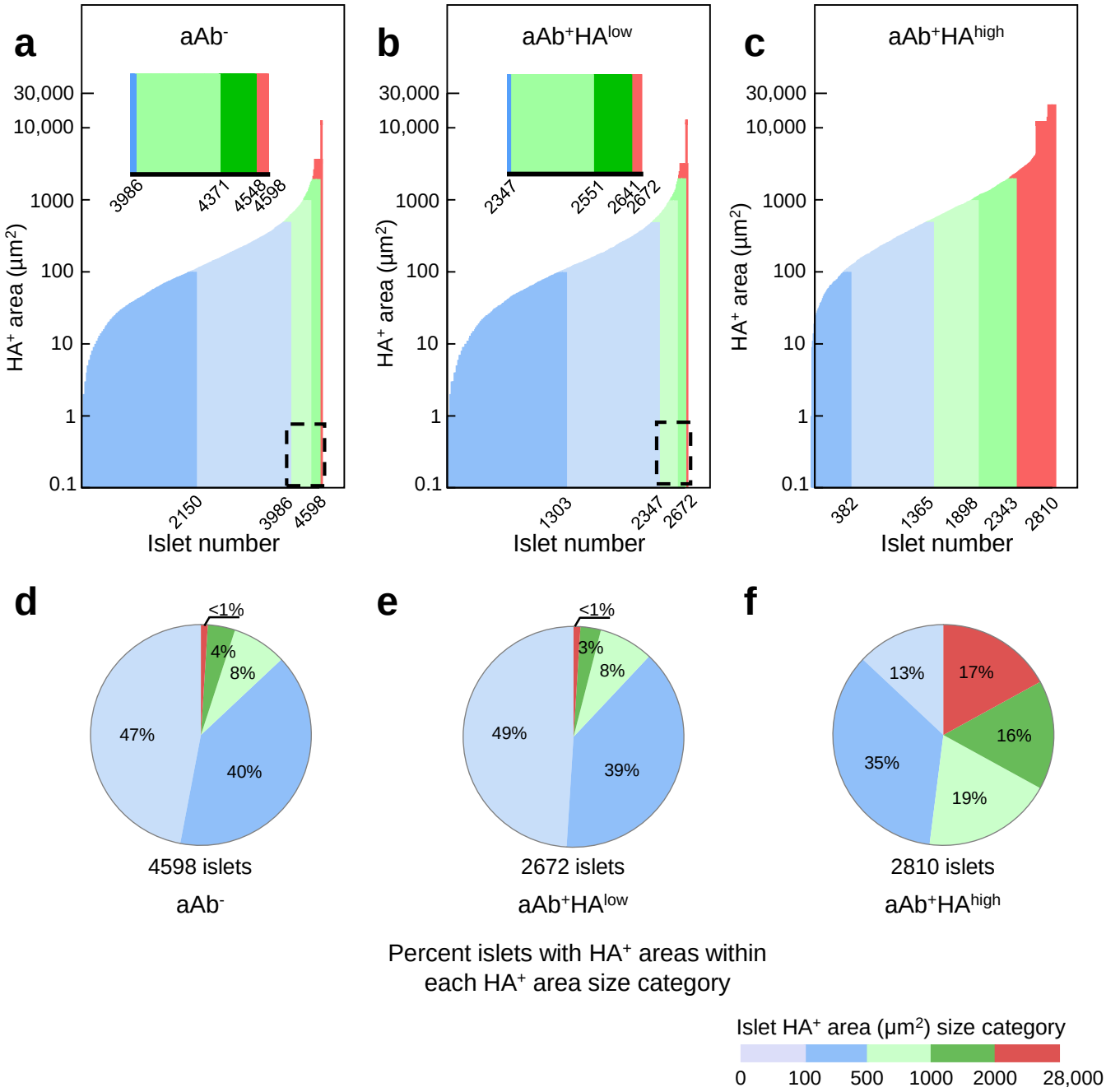
Note 1. The case numbers indicate the tissues from autoantibody-positive and autoantibody-negative donors, which are ranked according to the size of their islet HA-positive areas, with 1 and 16 having the most HA in their respective groups.
Note 2. Numbers 1 - 7 are the aAb$^+$HA^{high} cases; numbers 8-15 are the aAb+HA^{low} cases.
Note 3. C-peptide levels are in ng/ml.

ESM Table 2. Antibodies used for immunohistochemistry.

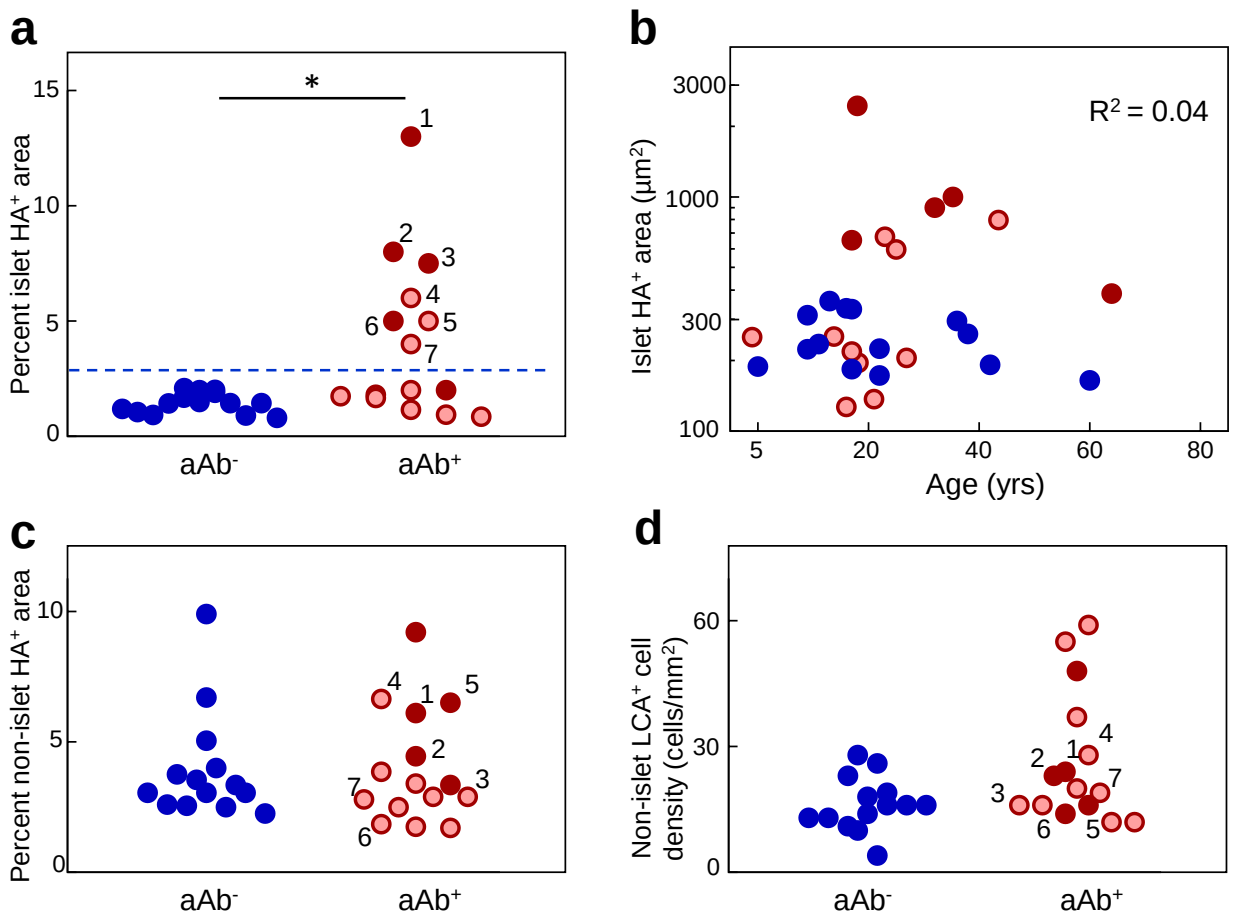
Antigen	Antibody supplier	Catalog number	Dilution
Primary antibodies			
Insulin	Abcam, Cambridge, MA, USA	ab7842	1:1000
Synaptophysin	Thermo Fisher, Waltham, MA, USA	MA5-11575	1:100
Ki67	Abcam, Cambridge, MA, USA	ab15580	1:100
CD3	DAKO, Carpinteria, CA, USA	A0452	1:100
CD11c	Abcam, Cambridge, MA, USA	ab52632	1:50
CD20	DAKO, Carpinteria, CA, USA	M0755	1:50
CD68	DAKO, Carpinteria, CA, USA	M0814	1:100
LCA	Abcam, Cambridge, MA, USA	ab187271	1:200
LCA (clone OX-1)	Bio-Rad, Hercules, CA, USA	MCA43	1:100
CD68 (clone ED1)	Bio-Rad, Hercules, CA, USA	MCA341	1:100
Secondary antibodies			
anti-guinea pig IgG	Jackson Immunoresearch laboratories, West Grove, PA, USA	706-065-148	1:500
anti-mouse IgG	Biocare Medical, Pacheco, CA, USA	M4U534	as indicated in the product datasheet
anti-rabbit IgG	Biocare Medical, Pacheco, CA, USA	M4U534	as indicated in the product datasheet
Antibody dilution buffer			
Phosphate buffer saline (PBS)	Thermo Fisher, Waltham, MA, USA	10010023	N/A

ESM Table 3. Insulitis grade, islet HA areas, and beta cell mass in BB rats.

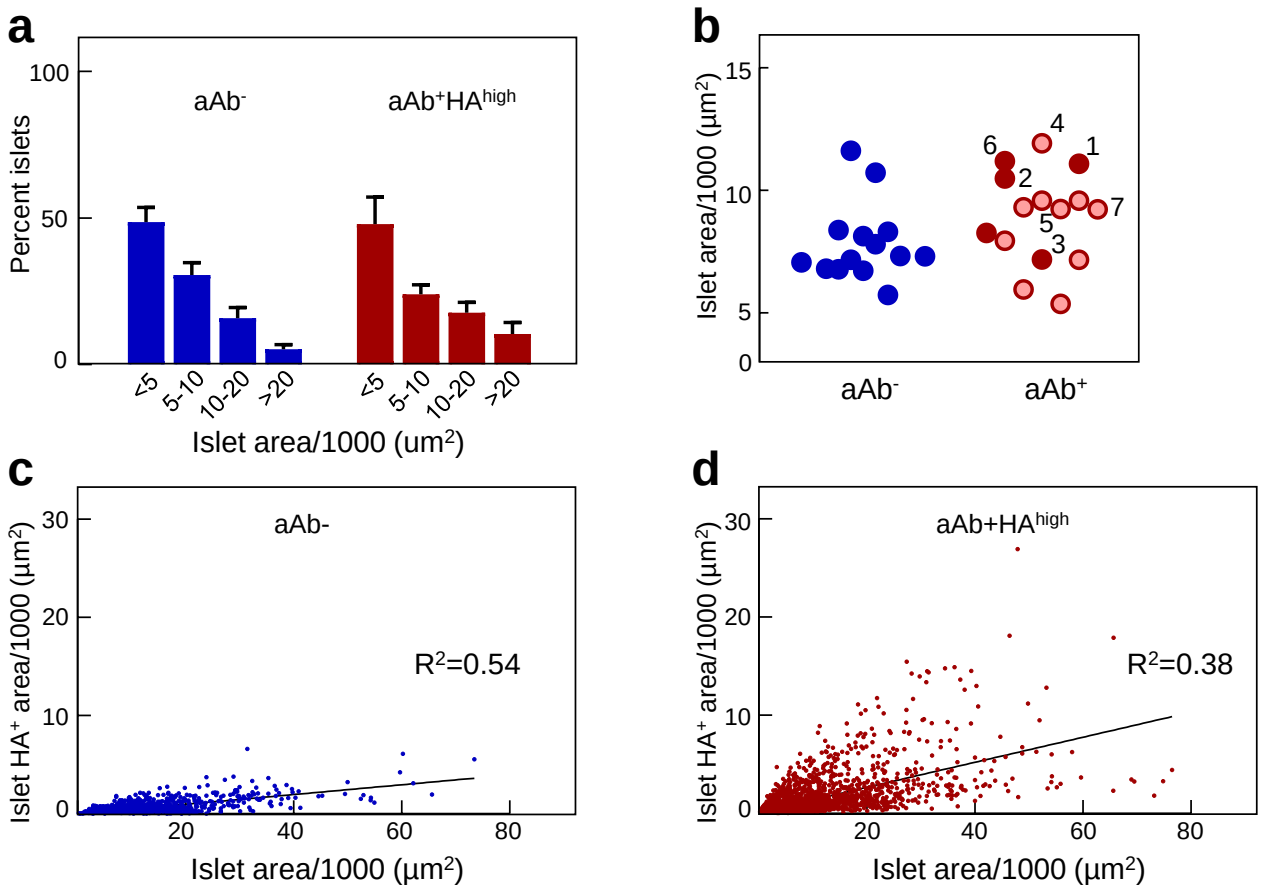
Genotype	Age (weeks)	n	Percent islets with insulitis grade				Overall Insulitis grade	Islet HA area (μm^2)	Beta cell mass (µg)
			0	1	2	3			
DR*Lyp*/+	7		100	0	0	0	0	960	1520
	7		100	0	0	0	0	1140	2380
	7		100	0	0	0	0	1070	2520
	7		100	0	0	0	0	910	1420
	8		100	0	0	0	0	1030	2100
	8		100	0	0	0	0	930	2700
	7-8	6	100	0	0	0	0	1010	2110
DR*Lyp/Lyp*	7		100	0	0	0	0	1400	2080
	7		100	0	0	0	0	2250	2650
	7		100	0	0	0	0	3860	1100
	7		100	0	0	0	0	1790	2010
	8		100	0	0	0	0	2540	1800
	8		100	0	0	0	0	2380	1910
	7-8	6	100	0	0	0	0	2370	1930
DR*Lyp/Lyp*	9		21	69	8	2	1	4190	950
	9		25	67	8	0	1	3730	1180
	9		26	72	2	0	1	4610	780
	9		26	63	10	1	1	4490	750
	10		16	73	11	0	1	7950	840
	10		25	68	6	1	1	4900	1120
	9-10	6	23	69	8	1	1	4980	940
DR*Lyp/Lyp*	10		4	9	79	8	2	9040	470
	11		8	28	50	14	2	5280	550
	11		13	5	66	16	2	9640	610
	11		3	25	62	10	2	6270	1050
	11		12	15	61	12	2	5890	1090
	10-11	5	8	16	64	12	2	7220	750
DR*Lyp/Lyp*	11		1	1	29	69	3	5620	130
	11		5	3	2	90	3	3320	230
	12		6	2	7	93	3	4620	170
	12		0	1	19	80	3	4100	490
	12		2	0	28	70	3	8120	260
	12		8	2	10	80	3	6550	630
	11-12	6	4	2	16	80	3	5390	320



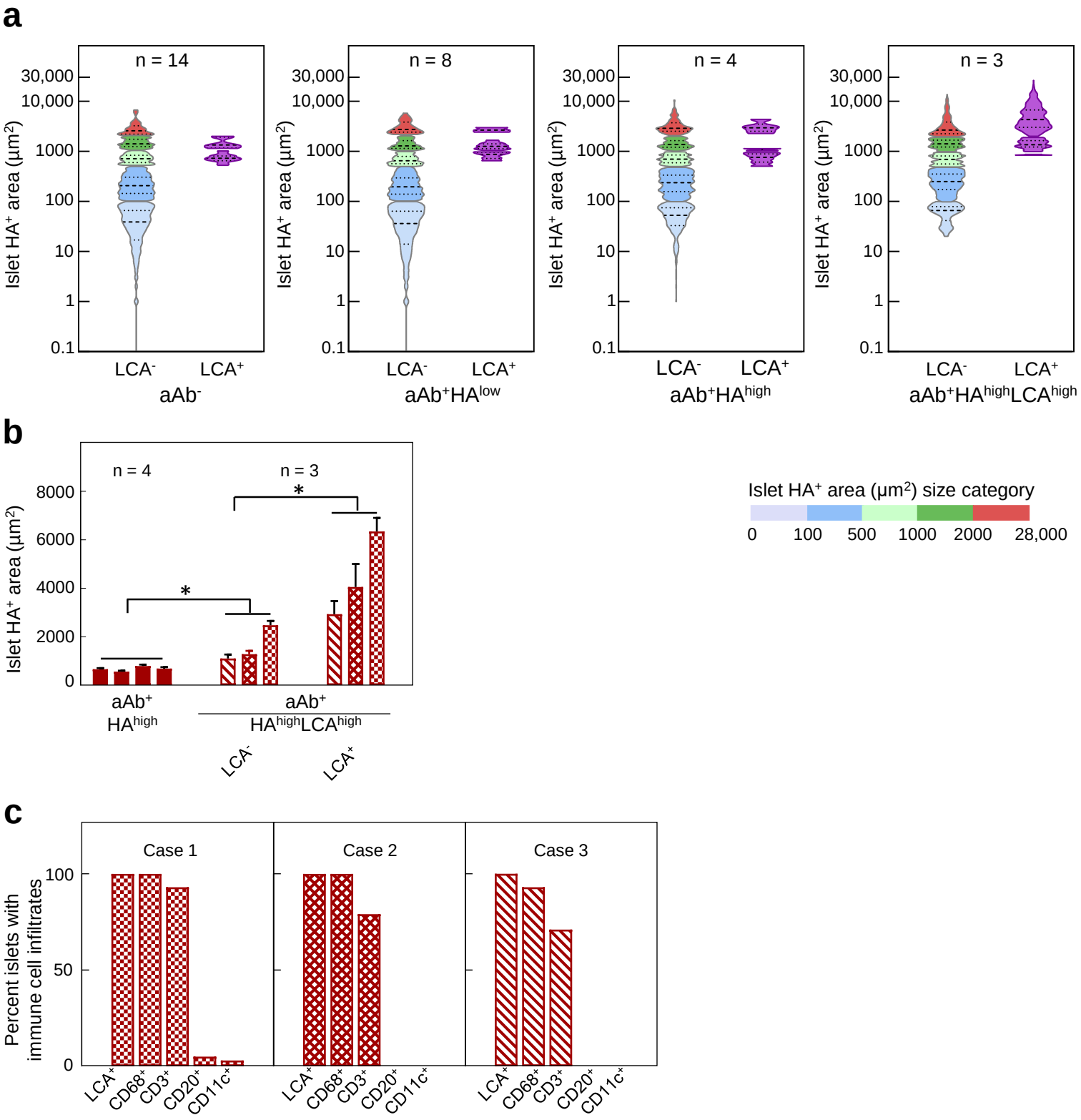
ESM Fig. 1 HA accumulates in islets in a subset of aAb$^+$ donors. (**a-c**) Histograms of individual HA$^+$ areas measured in 4598, 2672, and 2810 islets from control aAb$^-$, aAb$^+$HA^{low}, and aAb$^+$HA^{high} tissues, respectively. The values of the HA$^+$ areas for the indicated number of islets are in ascending order. The islet HA$^+$ areas are shown on a log10 scale. The numbers on the x-axis indicate the cumulative number of islets. (**a, b**) The cumulative numbers of islets with HA$^+$ areas within the 500-1000 μm^2, 1001-2000 μm^2, or >2000 μm^2 size categories, presented in the light green, dark green, and red bars, respectively, (insets) are shown magnified. (**d-f**) Islet HA$^+$ area size distribution. p<0.0005, one-way ANOVA, aAb$^+$HA^{high} vs. control or aAb$^+$HA^{low} tissues.



ESM Fig. 2 (**a**) Scatter plot of percent islet HA^+ areas. *p<0.0001, Mann-Whitney U test. The dotted line indicates the upper cut-off value (mean + 3SD) of the measurements obtained from the aAb^- controls. 4598, 2672, and 2810 islets from aAb^- control, aAb^+HA^{low}, and aAb^+HA^{high} tissues were analyzed, respectively. (**b**) Islet HA^+ area as a function of donor age. HA^+ areas (**c**) and LCA^+ cell density (**d**) in the non-islet region. Each circle denotes an individual donor. The islet HA^+ areas are shown on a log10 scale. Blue circles, aAb^- donors; light red circles, single-aAb^+; dark red circles, double-aAb^+.

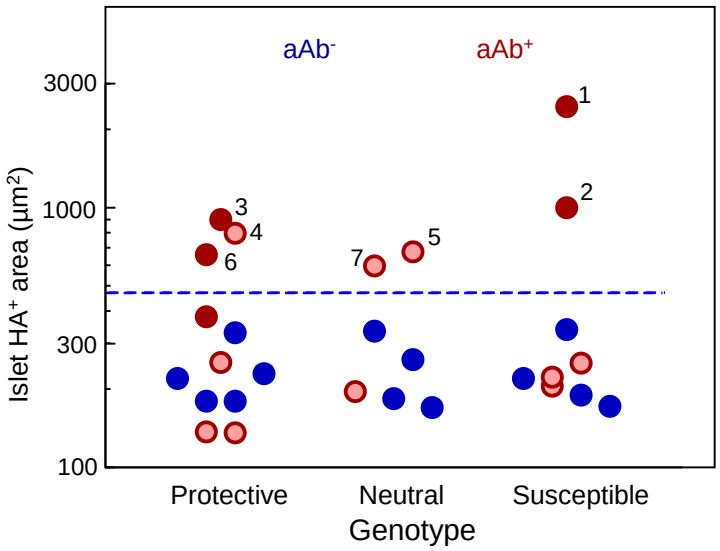


ESM Fig. 3 (**a**) Islet size distribution in 14 aAb$^-$ and 7 aAb$^+$HA^{high} tissues. (**b**) Islet areas in each individual tissue. Islet HA$^+$ area as a function of islet area in 14 aAb$^-$ (**c**) and 7 aAb$^+$HA^{high} (**d**) tissues. Each circle denotes an individual donor in (**b**) and an individual islet in (**c** and **d**). Blue bars and circles, aAb$^-$ donors; light red circles, single-aAb$^+$; dark red bars, aAb$^+$; dark red circles, double-aAb$^+$ in (**b**) and aAb$^+$HA^{high} in (**d**).

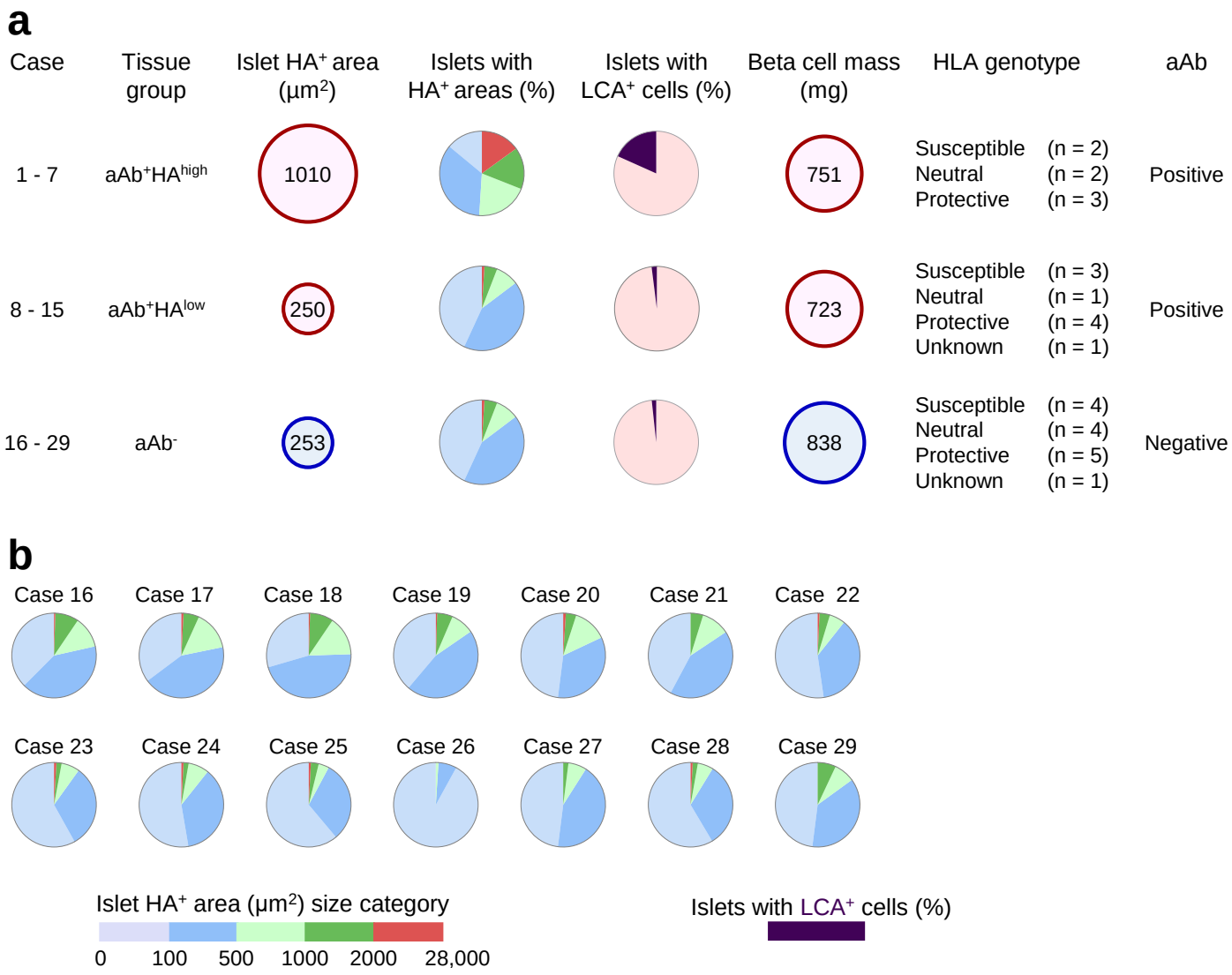


ESM Fig. 4 LCA$^+$ leukocytes infiltrate islets exclusively in regions containing the largest HA deposits.

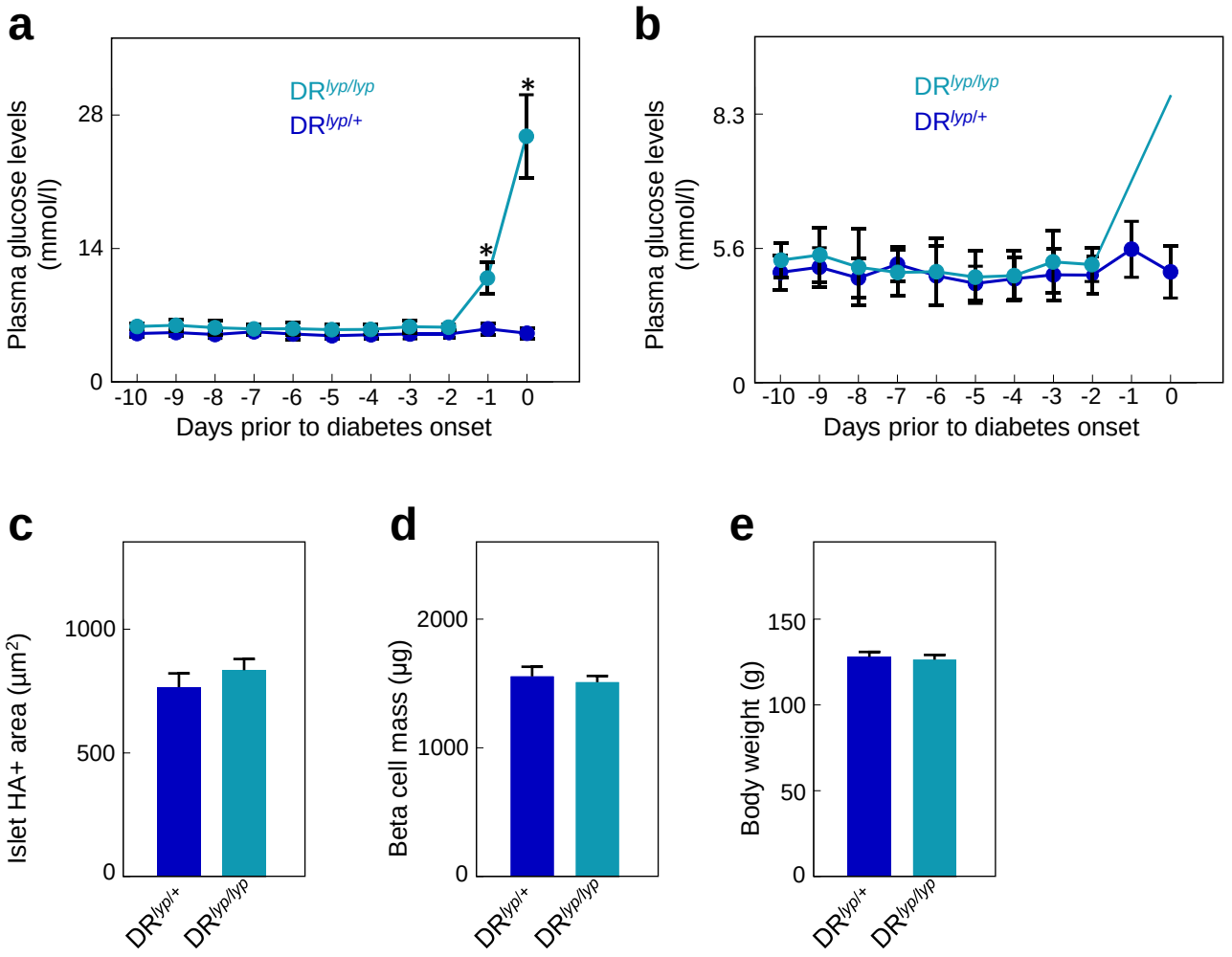
(**a**) Violin plots of HA$^+$ areas in islets without (LCA$^-$) or with (LCA$^+$) immune cells distributed within each HA area size category. The islet HA$^+$ areas are shown on a log10 scale. (**b**) Islet HA$^+$ areas in the 7 individual aAb$^+$HA^{high} tissues. Each bar represents one tissue. Solid bars, 4 aAb$^+$HA^{high} tissues which showed no evidence of insulitis; hatched bars, 3 aAb$^+$HA^{high} tissues with insulitis. In the 3 aAb$^+$HA^{high}LCA^{high} tissues, immune cell-free islets (LCA$^-$) and islets with associated immune cells (LCA$^+$) are assessed separately. Data are mean ± SEM of the measurements. *p<0.001, Mann-Whitney U test. (**c**) Proportion of islets with LCA$^+$ cell infiltrates containing CD68$^+$, CD3$^+$, CD20$^+$, and CD11c$^+$ cells.



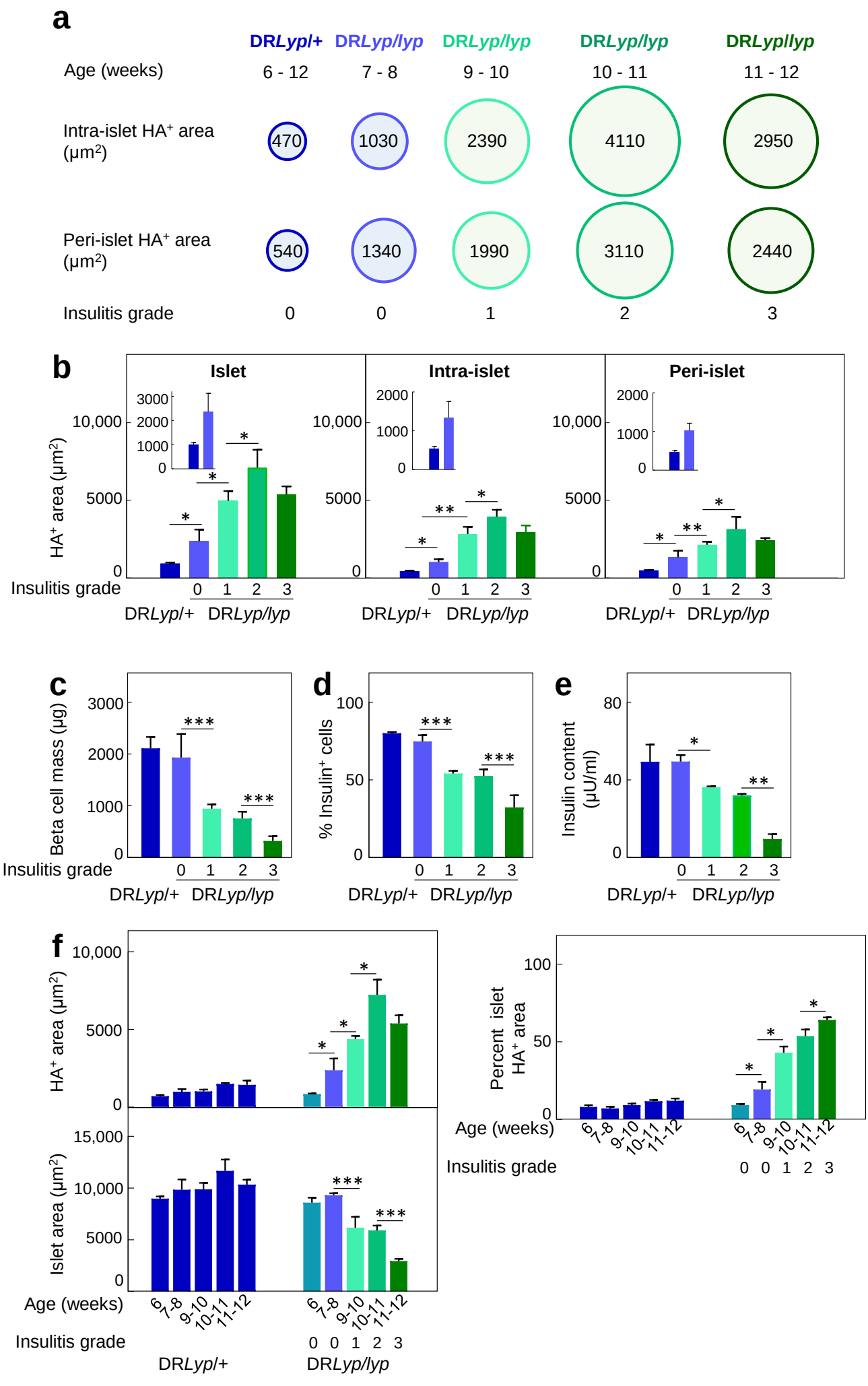
ESM Fig. 5 Scatter plot of islet HA^+ areas as a function of donor HLA genotypes associated with type 1 diabetes**.** Each circle denotes an individual donor. The islet HA^+ areas are shown on a log10 scale. Blue circles, aAb^- donors; light red circles, single-aAb^+; dark red circles, double-aAb^+. Data are mean values of HA^+ areas for each individual donor. The dotted line indicates the upper cut-off value (mean + 3SD) of the measurements obtained from the aAb^- controls. The numbers 1-7 indicate the aAb^+HA^{high} tissues ranked according to the size of their islet HA^+ areas.



ESM Fig. 6 (**a**) Islet HA$^+$ areas, insulitis, and beta cell mass in tissues from all donors. Data are mean values of measurements made in the 7 aAb$^+$HA^{high}, 8 aAb$^+$HA^{low}, and 14 control aAb$^-$ tissues. The pie charts represent the mean percentage of islets with HA$^+$ areas falling within each of the HA$^+$ areas size categories or the percentage of islets with LCA$^+$ cells. The size of the red or blue circles is proportional to the average size of the islet HA$^+$ areas or beta cell mass, which is indicated by the value number within the circle. The measurements made in islets from the individual aAb$^+$ donors are shown in Fig. 6. In the tissues from the 14 aAb$^-$ donors: Size range of islet HA$^+$ areas, 172 to 375 µm^2; range of proportion of islets with HA$^+$ areas, 38 to 59% for ≤100 µm^2, 31 to 52% for 101-500 µm^2, 3 to 13% for 501-1000 µm^2, 2 to 8% for 1001-2000 µm^2, and 0 to <1% for >2000 µm^2; range of beta cell mass, 390 mg to 1350 mg. (**b**) Islet HA$^+$ area size distribution in the aAb$^-$ control group. The pie charts represent the percentage of islets with HA$^+$ areas falling within each of the HA$^+$ area size categories.



ESM Fig. 7 Assessment of morphologic and metabolic parameters in BB rats. (**a**) Fed blood glucose levels in diabetes-resistant DR$^{lyp/+}$ and diabetes-prone DR$^{lyp/lyp}$ rats. Data are mean ± SD of measurements from 10-20 rats. *p<0.001, vs previous time point; Kruskal-Wallis test. (**b**) The blood glucose values in the two groups before the onset of hyperglycemia in the DR$^{lyp/lyp}$ rats are shown using smaller intervals on the y-axis. (**c**) Islet HA+ areas, (**d**) beta cell mass, and (**e**) body weight in DR$^{lyp/+}$ (blue bars) and DR$^{lyp/lyp}$ (light blue bars) rats at 6 weeks of age. Data are mean ± SD of individual measurements obtained from 20 rats in each group.


a
DRLyp/+
DRLyp/lyp
DRLyp/lyp
DRLyp/lyp
DRLyp/lyp
Age (weeks)
6 - 12
7 - 8
9 - 10
10 - 11
11 - 12
Intra-islet HA+ area (μm2)
470
1030
2390
4110
2950
Peri-islet HA+ area (μm2)
540
1340
1990
3110
2440
Insulitis grade
0
0
1
2
3
b
Islet
Intra-islet
Peri-islet
HA+ area (μm2)
Insulitis grade
DRLyp/+
DRLyp/lyp
c
Beta cell mass (μg)
d
% Insulin+ cells
e
Insulin content (μU/ml)
f
HA+ area (μm2)
Islet area (μm2)
Percent islet HA+ area
Age (weeks)

ESM Fig. 8 HA accumulates in islets while beta cell mass decreases in presymptomatic DR*Lyp/lyp* rats during the progression to hyperglycemia. (**a**) Intra- and peri-islet HA+ areas in diabetes-resistant DR*Lyp/*+ (blue circles) or diabetes-prone DR*Lyp/lyp* (light blue and green circles) rats exhibiting different degrees of insulitis. Data are the mean values of measurements obtained from 300-400 islets (5-6 rats) per group. The size of each circle is proportional to the average size of the HA+ areas which is indicated by the value number within the circle. (**b**) Islet, intra- and peri-islet HA+ areas in tissues from DR*Lyp*/+ (blue bars) or DR*Lyp/ly*p (light blue and green bars) rats. Left panel, data represent mean ± SEM of the measurements shown in Fig. 7k; middle and right panels, mean ± SEM of the measurements shown in (**a**) of this figure. (**c**) Beta cell mass, (**d**) percentage of insulin$^+$ cells, and (**e**) pancreas insulin content in DR*Lyp*/+ and DR*Lyp/ly*p rats. (**f**) Islet HA areas and islet areas in DR*Lyp*/+ and DR*Lyp/ly*p rats. Data are mean ± SEM of the measurements obtained from 5-6 rats per group. **p*<0.05, ***p*<0.01, ****p*<0.001 as indicated, Mann-Whitney *U* test.