Supplementary Materials for

Transplant tissue specific exosome platform for noninvasive monitoring of transplant organ rejection

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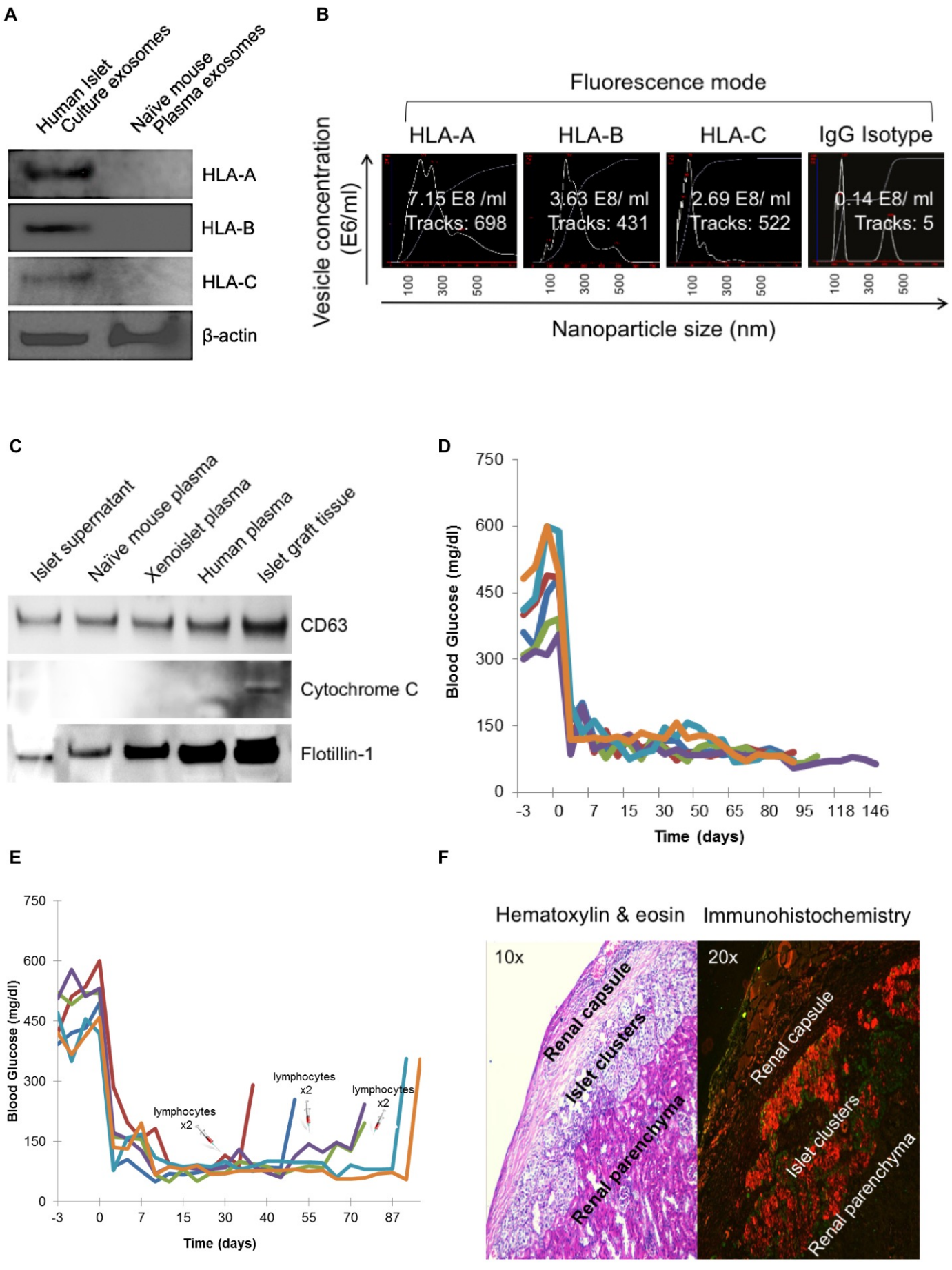
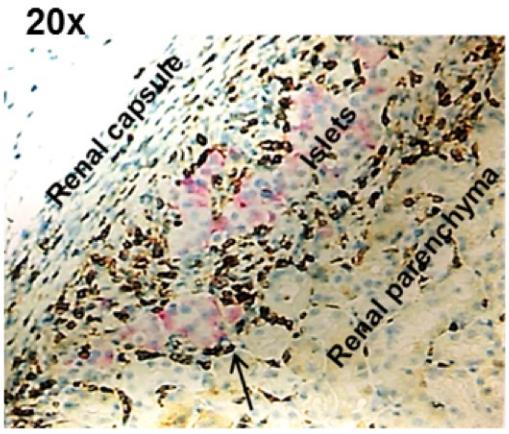

A
Human Islet Culture exosomes
Naïve mouse Plasma exosomes
HLA-A
HLA-B
HLA-C
β-actin
B
Fluorescence mode
HLA-A
HLA-B
HLA-C
IgG Isotype
Vesicle concentration (E6/ml)
7.15 E8 /ml
Tracks: 698
3.63 E8/ ml
Tracks: 431
2.69 E8/ ml
Tracks: 522
0.14 E8/ ml
Tracks: 5
100
300
500
Nanoparticle size (nm)
C
Islet supernatant
Naïve mouse plasma
Xenoislet plasma
Human plasma
Islet graft tissue
CD63
Cytochrome C
Flotillin-1
D
Blood Glucose (mg/dl)
750
600
450
300
150
0
-3
0
7
15
30
50
65
80
95
118
146
Time (days)
E
Blood Glucose (mg/dl)
750
600
450
300
150
0
lymphocytes x2
lymphocytes x2
lymphocytes x2
-3
0
7
15
30
40
55
70
87
Time (days)
F
Hematoxylin & eosin
Immunohistochemistry
10x
20x
Renal capsule
Islet clusters
Renal parenchyma
Renal capsule
Islet clusters
Renal parenchyma

G



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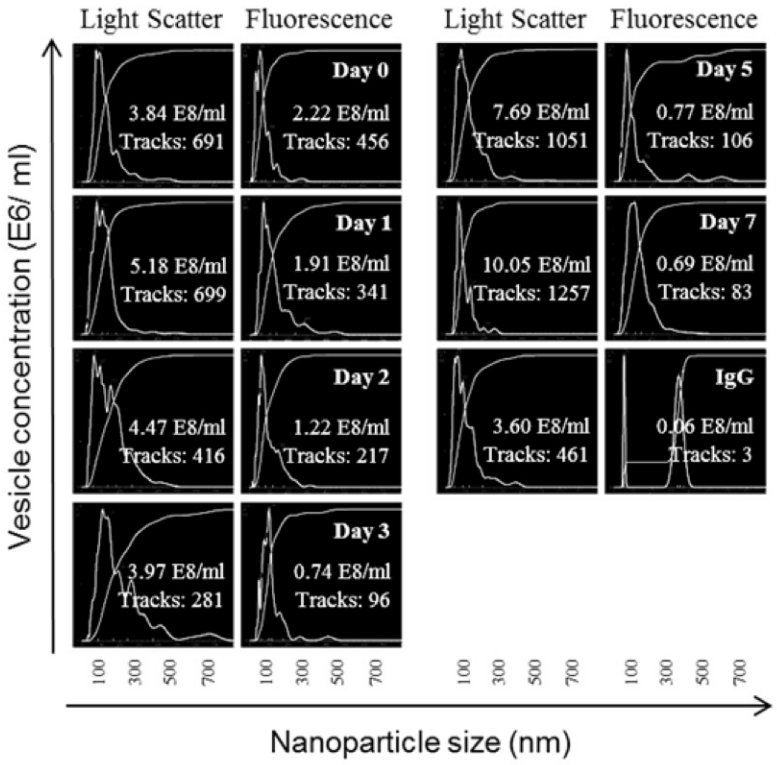

I

Time point	Mean C peptide level (ng/ ml)
Day 1	2.83
Day 2	3.14
Day 3	1.58
Day 5	0.78

Fig. S1. Xenoislet transplantation model. (A) Western blot analysis of exosomes isolated from supernatants of *in vitro* cultures of human pancreatic islets and naive, athymic mouse (recipient animal) plasma exosomes for human MHC class I molecules, HLA-A, HLA-B, and HLA-C is shown, along with β-actin control. HLA molecules were only detected in the human islet exosomes. (**B**) NanoSight fluorescence images of exosomes from human pancreatic islet culture are shown for human MHC molecules, HLA-A, HLA-B, and HLA-C. IgG isotype control is also shown. Islet exosomes express all three HLA class I molecules on their surface. (**C**) Extracellular microvesicles isolated using the described methodology were analyzed on Western blot for presence of exosome markers CD63 and flotillin-1, and for the absence of apoptotic body marker cytochrome c. The isolated samples showed enrichment of exosomes, without contamination from cellular particles/ apoptotic bodies. (**D**) Representative plasma fasting blood glucose values are shown for 6 xenoislet recipients. Day -3 represents values post-induction of diabetes with streptozotocin, before islet transplantation. Day 0 represents the time point of islet transplantation. All animals maintained normoglycemia in the post-transplantation period. (**E**) In 15 xenoislet recipients that remained normoglycemic post-transplant, recipient strain matched syngeneic luekocytes were injected intraperitoneally ($2x10^7$ cells/ animal) into the recipient, and daily blood glucose was tracked. The first day that glucose increased to $\geq$200 mg/dl, the animal was sacrificed for analysis of plasma exosome pool and for histological confirmation of immune rejection of the transplant islet mass. Representative glucose curves in 6 animals are shown, with the time of leukocyte injection labeled as lymphocytes. (**F**) Representative histology of the transplanted islet mass is shown from N-xeno animal. Hematoxylin & eosin staining showed viable islet clusters under the renal capsule, with immunohistochemistry confirming presence of insulin (red) and glucagon (green) inside the islet cells. (**G**) Histology of the transplanted islet mass from R-xeno animal is shown (Day 8 post syngeneic leukocyte infusion).

Immunohistochemistry for insulin (red) showed decreased islet mass underneath the renal capsule, along with dense infiltration into the islet graft by T cells (marked by arrow). Staining for T cells was performed using anti-CD3 antibody (brown). (**H**) Representative NanoSight panels for plasma HLA exosome signal during the period of acute rejection are shown for Day 0, 1, 2, 3, 5, and 7 post-infusion of donor sensitized leukocytes into the xenoislet recipients. IgG isotype control from Day 0 is also shown. (**I**) Mean plasma human specific C peptide values post-glucose stimulation during the acute rejection period for Day 1, 2, 3, and 5 post-leukocyte infusion are shown. Plasma collected after performing intraperitoneal glucose tolerance test was used for C peptide analysis. Even though the stimulated C peptide values decreased during the rejection period, they remained in the normal range.

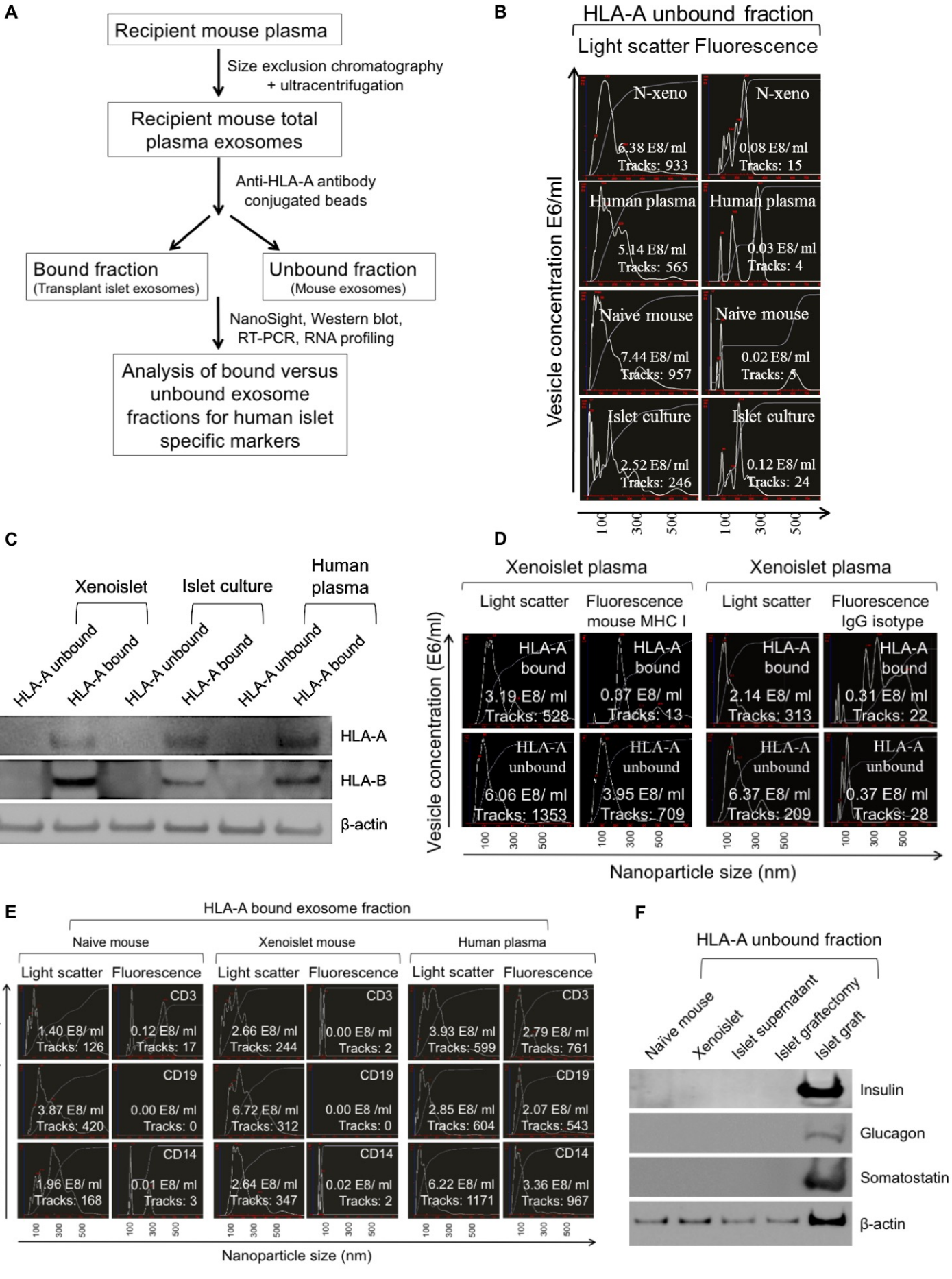
A
Recipient mouse plasma
Size exclusion chromatography + ultracentrifugation
Recipient mouse total plasma exosomes
Anti-HLA-A antibody conjugated beads
Bound fraction (Transplant islet exosomes)
Unbound fraction (Mouse exosomes)
NanoSight, Western blot, RT-PCR, RNA profiling
Analysis of bound versus unbound exosome fractions for human islet specific markers
B
HLA-A unbound fraction
Light scatter Fluorescence
Vesicle concentration E6/ml
N-xeno
6.38 E8/ ml
Tracks: 933
N-xeno
0.08 E8/ ml
Tracks: 15
Human plasma
5.14 E8/ ml
Tracks: 565
Human plasma
0.03 E8/ ml
Tracks: 4
Naive mouse
7.44 E8/ ml
Tracks: 957
Naive mouse
0.02 E8/ ml
Tracks: 5
Islet culture
2.52 E8/ ml
Tracks: 246
Islet culture
0.12 E8/ ml
Tracks: 24
100
300
500
C
Xenoislet
Islet culture
Human plasma
HLA-A unbound
HLA-A bound
HLA-A unbound
HLA-A bound
HLA-A unbound
HLA-A bound
HLA-A
HLA-B
β-actin
D
Xenoislet plasma
Xenoislet plasma
Light scatter
Fluorescence mouse MHC I
Light scatter
Fluorescence IgG isotype
Vesicle concentration (E6/ml)
HLA-A bound
3.19 E8/ ml
Tracks: 528
HLA-A bound
0.37 E8/ ml
Tracks: 13
HLA-A bound
2.14 E8/ ml
Tracks: 313
HLA-A bound
0.31 E8/ ml
Tracks: 22
HLA-A unbound
6.06 E8/ ml
Tracks: 1353
HLA-A unbound
3.95 E8/ ml
Tracks: 709
HLA-A unbound
6.37 E8/ ml
Tracks: 209
HLA-A unbound
0.37 E8/ ml
Tracks: 28
100
300
500
Nanoparticle size (nm)
E
HLA-A bound exosome fraction
Naive mouse
Xenoislet mouse
Human plasma
Light scatter
Fluorescence
Vesicle concentration (E6/ml)
CD3
1.40 E8/ ml
Tracks: 126
CD3
0.12 E8/ ml
Tracks: 17
CD3
2.66 E8/ ml
Tracks: 244
CD3
0.00 E8/ ml
Tracks: 2
CD3
3.93 E8/ ml
Tracks: 599
CD3
2.79 E8/ ml
Tracks: 761
CD19
3.87 E8/ ml
Tracks: 420
CD19
0.00 E8/ ml
Tracks: 0
CD19
6.72 E8/ ml
Tracks: 312
CD19
0.00 E8 /ml
Tracks: 0
CD19
2.85 E8/ ml
Tracks: 604
CD19
2.07 E8/ ml
Tracks: 543
CD14
1.96 E8/ ml
Tracks: 168
CD14
0.01 E8/ ml
Tracks: 3
CD14
2.64 E8/ ml
Tracks: 347
CD14
0.02 E8/ ml
Tracks: 2
CD14
6.22 E8/ ml
Tracks: 1171
CD14
3.36 E8/ ml
Tracks: 967
100
300
500
Nanoparticle size (nm)
F
HLA-A unbound fraction
Naïve mouse
Xenoislet
Islet supernatant
Islet graftectomy
Islet graft
Insulin
Glucagon
Somatostatin
β-actin

Fig. S2. TISE analysis in the xenoislet transplantation model. (**A**) Schematic of TISE enrichment is shown. Exosomes were isolated from mouse plasma using size exclusion chromatography and ultracentrifugation. TISE were enriched from total plasma exosome pool using anti-human MHC class I specific antibody conjugated magnetic beads (anti-HLA-A antibody). The bead bound fraction representing TISE was then analyzed for expression of islet speicific markers, and for characterization of its protein and RNA cargo. The unbound fraction representing exosome contribution from recipient mouse tissues was analyzed to confirm specificity and sensitivity of the TISE purification protocol. (**B**) NanoSight analysis of HLA-A unbound exosome fractions for HLA-A is shown. On fluorescence mode, HLA-A positive exosomes were undetectable in the unbound fraction samples from xenoislet mouse plasma, naive mouse plasma, human plasma, and human islet culture supernatant. (**C**) Western blot analysis of the HLA-A bound and HLA-A unbound exosome fractions for HLA-A protein is shown for xenoislet plasma, human islet culture supernatant, and human plasma. HLA-A expression was noted in all the HLA-A bound fractions, but not in the HLA-A unbound fractions. (**D**) Xenoislet plasma from HLA-A bound and unbound exosome fractions for presence of mouse tissue specific exosomes was analyzed using anti-mouse MHC class I specific antibody quantum dot. Mouse MHC class I signal was undetectable in the HLA-A bound fraction, but was highly expressed in the HLA-A unbound fraction. IgG isotype control is also shown. (**E**) HLA-A bound exosomes were analyzed on NanoSight fluorescence for presence of leukocyte derived exosomes using antibodies against leukocyte specific surface markers: anti-human CD3 for T cell, anti-human CD19 for B cell, and anti-human CD14 for monocyte. HLA-A bound exosome fractions obtained from naive mouse plasma, xenoislet plasma (N-xeno), and naive human plasma were analyzed for the leukocyte markers. Both naive mouse and xenoislet mouse plasma HLA-A bound exosomes did not show expression of leukocyte markers, but the naive human plasma HLA-A bound exosomes showed high co-expression of human leukocyte markers. This suggests that the majority of the HLA-A bound exosomes in the xenoislet plasma are not from a major contribution from passenger human leukocytes. (**F**) Western blot analysis of HLA-A unbound fractions for presence of islet endocrine hormones insulin, glucagon, and somatostatin, is shown. In naive mouse plasma, xenoislet plasma, human islet culture supernatant, and in plasma from xenoislet animal after islet graftectomy, islet endocrine hormones were undetectable. Islet graft tissue is shown as positive control, along with β-actin control.

Supplementary Table 1. Proteomic profile of TISE enriched from N-xeno xenoislet plasma. Expression data as analyzed with PD/ Scaffold software package is shown for all proteins with cut-off values: peptide p-value <95%, protein p-value <99%.

Protein	**Expression level (total spectral counts)**
Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1	39
Ugl-Y3 OS=Homo sapiens GN=FN1 PE=2 SV=1	28
Collagen alpha-1(I) chain OS=Homo sapiens GN=COL1A1 PE=1 SV=5	13
Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2	13
Junction plakoglobin OS=Homo sapiens GN=JUP PE=2 SV=1	9
Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3	7
Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2	7
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=2 SV=1	7
Isoform 2 of Angiopoietin-1 OS=Homo sapiens GN=ANGPT1	6
Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1	5
Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=2	4
Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2	4
Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3	4
Ig kappa chain V-IV region (Fragment) OS=Homo sapiens GN=IGKV4-1 PE=4 SV=1	4
Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	3
Collagen alpha-2(I) chain OS=Homo sapiens GN=COL1A2 PE=1 SV=7	3
Hemoglobin subunit delta OS=Homo sapiens GN=HBD PE=2 SV=1	3
Vimentin OS=Homo sapiens GN=VIM PE=3 SV=1	3
Protein tyrosine phosphatase, receptor type, D, isoform CRA_c OS=Homo sapiens GN=PTPRD PE=4 SV=1	3
Ig heavy chain V-III region GAL OS=Homo sapiens PE=1 SV=1	3
Histone H2A type 1-B/E OS=Homo sapiens GN=HIST1H2AB PE=1 SV=2	3
Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=2 SV=1	3
Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1	3
Haptoglobin OS=Homo sapiens GN=HP PE=2 SV=4	2
Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	2
Ig kappa chain V-II region Cum OS=Homo sapiens PE=1 SV=1	2
Myosin regulatory light chain 12A OS=Homo sapiens GN=MYL12A PE=4 SV=1	2
Keratin, type II cytoskeletal 78 OS=Homo sapiens GN=KRT78 PE=2 SV=2	2

Trypsin-1 OS=Homo sapiens GN=PRSS1 PE=2 SV=1	2
Myosin light chain 3 OS=Homo sapiens GN=MYL3 PE=1 SV=3	2
Thrombospondin-1 OS=Homo sapiens GN=THBS1 PE=1 SV=2	1
Isoform Er9 of Ankyrin-1 OS=Homo sapiens GN=ANK1	1
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	1
Histone H2B OS=Homo sapiens GN=HIST2H2BF PE=2 SV=1	1
35 kDa inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=2 SV=1	1
Apolipoprotein C-I OS=Homo sapiens GN=APOC1 PE=1 SV=1	1
Ig kappa chain V-I region Lay OS=Homo sapiens PE=1 SV=1	1
Filaggrin-2 OS=Homo sapiens GN=FLG2 PE=1 SV=1	1
Collagen alpha-1(III) chain OS=Homo sapiens GN=COL3A1 PE=1 SV=4	1
Complement C1q subcomponent subunit B OS=Homo sapiens GN=C1QB PE=1 SV=3	1
Alpha-crystallin B chain OS=Homo sapiens GN=CRYAB PE=1 SV=2	1
Filamin-A OS=Homo sapiens GN=FLNA PE=2 SV=1	1
Ubiquitin OS=Homo sapiens GN=UBB PE=2 SV=1	1
Oncoprotein-induced transcript 3 protein OS=Homo sapiens GN=OIT3 PE=1 SV=2	1
Aldehyde dehydrogenase family 16 member A1 OS=Homo sapiens GN=ALDH16A1 PE=2 SV=1	1
Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	0
Isoform 3 of Histone-lysine N-methyltransferase 2C OS=Homo sapiens GN=KMT2C	0
Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2	0
Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	0
Isoform 2 of Liver carboxylesterase 1 OS=Homo sapiens GN=CES1	0
Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2	0
Talin-1 OS=Homo sapiens GN=TLN1 PE=2 SV=1	0
Complement C1r subcomponent OS=Homo sapiens GN=C1R PE=1 SV=2	0

Fig. S3. Donor HLA class I specific exosome profiles over long term follow-up in patients undergoing allogeneic islet transplantation. Plasma samples from islet transplant recipients, patients A, B, C, and D, were analyzed on the NanoSight for donor islet HLA class I specific exosome detection and quantitation. Light scatter and fluorescence signals for patients A (HLA-B13), B (HLA-A2), C (HLA-B8), and D (HLA-B8) over long term follow-up are shown. In all four patients, pretransplant fluorescence for donor HLA class I was similar to the post-transplant IgG isotype control. Time point of analysis (minutes (min) or days (d#) post-transplant) is shown in each NanoSight panel, with follow-up ranging from 60 minutes to 1848 days post-islet transplantation. Transplant islet exosome signal was reliably tracked in all islet transplant patients, similar to that seen in the xenoislet model.

Supplementary Table 2. Clinical data, TISE signal, and donor-recipient HLA profiles for Patient A is shown.

Interval (days post-transplant)	TISE signal	C-peptide (ng/ml)	Glucose (mg/dl)	C peptide to glucose ratio [C-peptide (ng/ml)/ glucose (mg/dl) * 100]	GAD65	Insulin (Units/day)
Pre transplant	0.05	0.0	162	0.03	0	29
0	0.68					
28		0.9	122	0.91		
56	0.54	0.6	96	0.59		
76		0.9	100	0.93	2	0
119		1.3	115	1.28		0
155		1.3	101	1.33	7	0
181		1.2	103	1.23		0
238		1.1	106	1.13		0
274		0.6	97	0.64	11	0
301		1.0	100	1.03		0
336		0.6	105	0.65		0
363		1.2	100	1.24	11	0
455	0.39					0
546		0.7	100	0.71	6	0
733		1.6	97	1.64	10	0
992		1.4	115	1.36		0
1001	0.25				125	0
1072		0.6	118	0.59		0
1098		0.2	105	0.24	668	12
1197	0.22	0.1	111	0.07		17
1288		0.1	160	0.06		
1302		0.1	227	0.11		20

Histocompatibility Report Patient A

	A	B	C	Bw	DRB1	DRw	DQB	DQA	DPB	DPA
Patient A	1	8	7	6	4	52	2			
	2	41	17	6	17	53	2			
Donor	2	7	6	4	7	53	2	0102		
	3	13	7	6	15	51	6	0201		

Supplementary Table 3. Clinical data, TISE signal, and donor-recipient HLA profiles for Patient B is shown.

Interval (days post-transplant)	TISE signal	C-peptide (ng/ml)	Glucose (mg/dl)	C peptide to glucose ratio [C-peptide (ng/ml)/ glucose (mg/dl) * 100]	GAD65	Insulin (Units/day)
Pre transplant	0.07	0.1	105	0.05	112	30
0	0.41	1.1	84	1.27		
51		0.8	76	1.05		
72		1.3	89	1.44	209	0
115		2.0	102	1.96		0
156		1.8	85	2.08		0
185	0.40	1.4	88	1.57	140	0
214		1.8	86	2.07		0
242		1.7	90	1.86		0
268		1.3	94	1.34	122	0
303		1.6	91	1.77		0
331		2.2	102	2.18		0
364		1.9	100	1.85	164	0
549		1.9	89	2.08	109	0
730		1.5	92	1.59	110	0
919					100	0
1018		1.7	92	1.85		0
1109	0.50	2.3	98	2.39	115	0
1194						0
1384		1.7	95	1.79		0
1529		2.6	98	2.66	217	0
1848	0.62	1.7	97	1.75	63	0

Histocompatibility Report Patient B

	A	B	C	Bw	DRB1	DRw	DQB	DQA	DPB	DPA
Patient B	3	18	5	6	4	52	2	03		
	68	60	7	6	17	53	8	05		
Donor	2	60	10	6	4	52	6	01		
	30	60	10	6	13	53	8	03		

Supplementary Table 4. Clincal data, TISE signal, and donor-recipient HLA profiles for Patient C is shown.

Interval (days post-transplant)	TISE signal	C-peptide (ng/ml)	Glucose (mg/dl)	C peptide to glucose ratio [C-peptide (ng/ml)/ glucose (mg/dl) * 100]	GAD65	Insulin (Units/day)
Pre transplant	0.02	0.1	169	0.03	3	49
0	0.35					
26		0.1	62	0.08		
52	0.42					
73		1.2	98	1.24	5	0
115		0.8	106	0.76		0
143		1.2	106	1.15		0
171		1.2	120	0.96	13	0
207		0.8	112	0.72		0
241		0.9	112	0.82		0
262	0.33	1.0	135	0.73	13	0
307		0.4	93	0.39		10
332		0.9	111	0.79		5
360		0.9	116	0.76	20	0
458		0.6	111	0.50		0
549		0.7	100	0.73	18	0
731		1.7	116	1.45	7	0
915					9	0
1006		0.4	93	0.43	12	0
1097	0.55	1.2	111	1.07	10	0
1476		0.5	120	0.43	7	0
1865		0.7	107	0.69	10	0

Histocompatibility Report Patient C

	A	B	C	Bw	DRB1	DRw	DQB	DQA	DPB	DPA
Patient C	1	37	5	4	4	52	3			
	2	44	6	4	11	53	3			
Donor	26	8	6	4	11	52	7	01		
	30	58	7	6	15	51	6	05		

Supplementary Table 5. Clincal data, TISE signal, and donor-recipient HLA profiles for Patient D is shown.

Interval (days post-transplant)	TISE signal	C-peptide (ng/ml)	Glucose (mg/dl)	C peptide to glucose ratio [C-peptide (ng/ml)/ glucose (mg/dl) * 100]	GAD65	Insulin (Units/day)
Pre transplant	0.03	0.1	102	0.05	0	27
0	0.39				5	
29		0.5	139	0.38		
40	0.43	0.6	105	0.60		
75		1.5	110	1.33	6	0
113		1.4	105	1.36		0
147		1.3	102	1.27		0
180		1.3	99	1.31	11	0
209		0.8	115	0.70		0
244		1.9	98	1.91		0
274		1.3	116	1.15	17	0
296		1.1	104	1.02		0
329		1.2	90	1.37		0
372	0.36	0.7	105	0.65	32	0
547		1.1	101	1.12	25	0
728		1.1	103	1.06	21	0
1004		1.1	110	1.01		0
1092	0.40	1.3	115	1.17	23	0
1367		0.8	97	0.78		0
1470	0.37	0.7	112	0.60	25	0
1834	0.44	1.6	148	1.09	35	0

Histocompatibility Report Patient D

	A	B	C	Bw	DRB1	DRw	DQB	DQA	DPB	DPA
Patient D	2	44	3	4	4	52	6			
	24	62	5	6	13	53	7			
Donor	3	7	7	6	15	52	2	0102		
	24	8	7	6	17	51	6	0501		

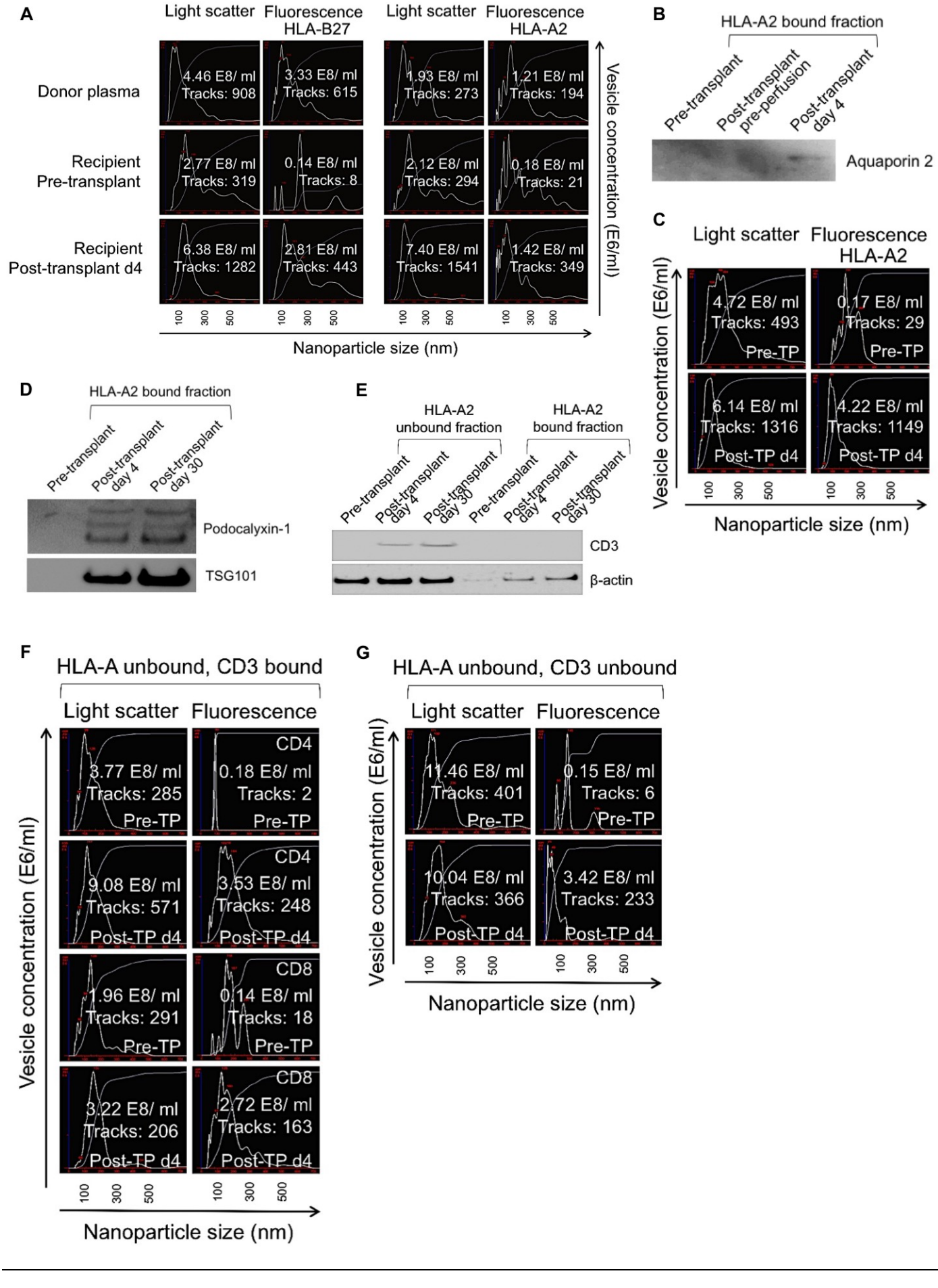
A
Light scatter
Fluorescence HLA-B27
Light scatter
Fluorescence HLA-A2
Donor plasma
4.46 E8/ ml Tracks: 908
3.33 E8/ ml Tracks: 615
1.93 E8/ ml Tracks: 273
1.21 E8/ ml Tracks: 194
Recipient Pre-transplant
2.77 E8/ ml Tracks: 319
0.14 E8/ ml Tracks: 8
2.12 E8/ ml Tracks: 294
0.18 E8/ ml Tracks: 21
Recipient Post-transplant d4
6.38 E8/ ml Tracks: 1282
2.81 E8/ ml Tracks: 443
7.40 E8/ ml Tracks: 1541
1.42 E8/ ml Tracks: 349
Vesicle concentration (E6/ml)
100 300 500
Nanoparticle size (nm)
B
HLA-A2 bound fraction
Pre-transplant
Post-transplant pre-perfusion
Post-transplant day 4
Aquaporin 2
C
Light scatter
Fluorescence HLA-A2
Vesicle concentration (E6/ml)
4.72 E8/ ml Tracks: 493 Pre-TP
0.17 E8/ ml Tracks: 29 Pre-TP
6.14 E8/ ml Tracks: 1316 Post-TP d4
4.22 E8/ ml Tracks: 1149 Post-TP d4
100 300 500
Nanoparticle size (nm)
D
HLA-A2 bound fraction
Pre-transplant
Post-transplant day 4
Post-transplant day 30
Podocalyxin-1
TSG101
E
HLA-A2 unbound fraction
HLA-A2 bound fraction
Pre-transplant
Post-transplant day 4
Post-transplant day 30
CD3
β-actin
F
HLA-A unbound, CD3 bound
Light scatter
Fluorescence
Vesicle concentration (E6/ml)
3.77 E8/ ml Tracks: 285 Pre-TP
CD4 0.18 E8/ ml Tracks: 2 Pre-TP
9.08 E8/ ml Tracks: 571 Post-TP d4
CD4 3.53 E8/ ml Tracks: 248 Post-TP d4
1.96 E8/ ml Tracks: 291 Pre-TP
CD8 0.14 E8/ ml Tracks: 18 Pre-TP
3.22 E8/ ml Tracks: 206 Post-TP d4
CD8 2.72 E8/ ml Tracks: 163 Post-TP d4
100 300 500
Nanoparticle size (nm)
G
HLA-A unbound, CD3 unbound
Light scatter
Fluorescence
Vesicle concentration (E6/ml)
11.46 E8/ ml Tracks: 401 Pre-TP
0.15 E8/ ml Tracks: 6 Pre-TP
10.04 E8/ ml Tracks: 366 Post-TP d4
3.42 E8/ ml Tracks: 233 Post-TP d4
100 300 500
Nanoparticle size (nm)

Fig. S4. Transplant tissue specific exosome platform can be studied in human renal transplantation. Donor kidney specific exosomes were characterized from recipient plasma and urine in human living donor renal transplantation. Representative analysis from 1 of 5 is shown (donor specific alleles: HLA-A2 and HLA-B27 positive). (**A**) Strong signals for HLA-A2 and HLA-B27 were seen in Donor (positive control) and Recipient post-transplant day 4 plasma samples, but not in Recipient pre-transplant sample ($p=0.008$; $n=5$). (**B**) HLA-A2 bound exosomes showed expression of aquaporin 2, renal tubule marker, in post-transplant day 4 sample, but not pre-transplant sample ($p=0.008$; $n=5$). Intraoperative recipient plasma obtained before organ perfusion (labeled post-transplant pre-perfusion) was also negative for aquaporin 2. (**C**) Recipient urine exosome analysis showed strong HLA-A2 signal on Post-transplant day 4, but not pre-transplant ($p=0.008$; $n=5$). (**D**) Western blot analysis of urine HLA-A2 bound exosomes showed co-expression of renal glomerular protein, podocalyxin-1, on post-transplant days 4 and 30, but not pre-transplant ($p=0.008$; $n=5$). (**E**) Urine HLA-A2 bound and unbound exosome fraction analysis for T cell marker, CD3, showed its presence only in the unbound fractions from post-transplant days 4 and 30 ($p=0.008$; $n=5$). (**F**) T cells exosomes, enriched using anti-CD3 antibody beads from HLA-A2 unbound fraction showed co-expression of helper T cell (CD4) and cytotoxic T cell (CD8) surface markers in the post-transplant day 4 sample but not the pre-transplant sample ($p=0.008$; $n=5$). (**G**) To assess feasibility of characterization of B cell exosomes from recipient urine, HLA-A2 unbound, CD3 unbound exosomes were analyzed for CD19 expression. Post-transplant day 4 sample (bottom panel) showed CD19 signal but not the pre-transplant sample ($p=0.008$; $n=5$).

Supplementary materials and methods

Mice

All the experiments were conducted in accordance to approved protocols through the University of Pennsylvania Institutional Animal Care and Use Committee, and in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health. Inbred nude, athymic mice (Nu/J strain) served as recipients of xenoislet transplants (Jackson Laboratory, Bar Harbor, ME).

Mouse islet transplantation and post-transplant monitoring

One week prior to xenoislet transplantation, nude mice were rendered diabetic through an intraperitoneal injection of streptozotocin (200 ug/kg) and confirmed for hyperglycemia >400 mg/dl over 3 days. Under general anesthesia (2-5% isoflurane, Primal Healthcare Ltd, India), through a lateral flank incision, 2000 islet equivalents (IE) of purified human islets were injected underneath the renal capsule of the nude mouse. The animal's glycemic status was monitored at least 3 times a week to ensure normoglycemia.

In xenoislet animals, to study if the HLA exosome signal was specific to the transplanted islet tissue, islet graftectomy was performed post-transplant under general anesthesia by reopening the flank incision. The kidney bearing the human islet transplant mass was removed en bloc upon ligation of renal vessels and the ureter. The animal was then monitored postoperatively to confirm recurrence of diabetic status (glucose >400 mg/dl). The transplanted islet mass was excised from the mouse renal capsule and RNA and protein were extracted from the transplant mass for microarray profiling, and to serve as islet tissue positive control in the Western blot and RT-PCR assays analyzing exosome cargo. To induce immune rejection in xenoislet animal (R-xeno), normoglycemic animal received intraperitoneal injection of syngeneic, NU/J strain wild type leukocytes ($2x10^7$ cells/ animal) that were presensitized with donor specific human islets. Animal's glycemic status was monitored daily, and the first time glucose increased >200 mg/dl the animal was sacrificed and plasma and islet graft were procured. For time course analysis of the kinetics of the HLA exosome signal during the earlier stages of acute rejection when the animal was still normoglycemic, xenoislet recipients were sacrificed at the following time points after infusion of donor sensitized syngeneic leukocytes: day 0 (4 hours post-infusion), 1, 2, 3, 5, and 7. All animals maintained normoglycemia until day 7 or 8 post-infusion. As control, xenoislet recipients received placebo infusion of 0.5 ml of PBS intraperitoneally and the animals were sacrificed at following time points post-infusion: day 0 (4 hours), 1, 2, and 3.

Intraperitoneal glucose tolerance test

After overnight fast xenoislet mice were given intraperitoneal injection of dextrose at 3 g/kg, and blood glucose was checked at following time points: 0, 15, 30, 60, and 90 minutes. Glucose tolerance tests were performed daily, including the day of sacrifice.

Human C-peptide ELISA

Mouse plasma samples were analyzed using human specific ultrasensitive C-peptide ELISA (Mercodia, Winston-Salem, NC) per manufacturer's protocol. Briefly, serum sample was added to a microtitration well with bound anti-human specific C-peptide . Using a direct sandwich technique, second peroxidase conjugated anti-C-peptide antibody was added and bound antibody was detected by addition of 3, 3',5,5'-tetramethylbenzidine. All samples were run in duplicate.

Immunohistochemistry

Islet graft tissue was cut with cryostat and fixed with 4% paraformaldehyde after washing with PBS. Blocking solution (0.05% Triton X-100) was added and then tissue slides were treated with primary antibodies to insulin (Santa Cruz Biotechnology, Santa Cruz, CA) and glucagon (Santa Cruz) overnight. For T cell detection in the R-xeno sample for time course analysis of acute rejection in xenoislet model, anti-CD3 antibody (Santa Cruz) was used. Slides were washed 3xPBS and the secondary antibody was added for detection. Analysis was performed using Zeiss epifluorescence microscope.

RNA microarray

Briefly, 50 to 100 ng of total RNA was converted to first- strand cDNA using reverse transcriptase primed by poly(T) and random oligomers that incorporated an RNA priming region. Second-strand cDNA synthesis was followed by ribo-SPIA linear amplification of each transcript using an isothermal reaction with RNase, RNA primer and DNA polymerase, and the resulting ssDNA was assessed by Bioanalyzer, fragmented and biotinylated by terminal transferase end labeling. Five and a half micrograms of labeled cDNA were added to Affymetrix hybridization cocktails, heated at 99oC for 5 min and hybridized for 16 hours at 45oC to human transcriptome 2.0 ST GeneChips (Affymetrix Inc., Santa Clara, CA) using the GeneChip Hybridization oven 645. The microarrays were then washed at low (6X SSPE) and high (100mM MES, 0.1M NaCl) stringency and stained with streptavidin-phycoerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycoerythrin stain. A GeneChip 3000 7G scanner was used to collect fluorescence signal l. Affymetrix Command Console and Expression Console were used to quantitate expression levels for targeted genes; default values provided by Affymetrix were applied to all analysis parameters.

Small RNA microarray analysis was performed at the Molecular Profiling Facility, University of Pennsylvania. Quality control tests of the total RNA samples were done by using the Agilent Bioanalyzer and Nanodrop spectrophotometry. Standard protocols were conducted as described in the Affymetrix FlashTag™ Biotin HSR RNA Labeling Kit manual and the Affymetrix GeneChip Expression Analysis Technical Manual. Briefly, RNA samples were submitted to a tailing reaction followed by ligation of the biotinylated signal molecule to the target RNA sample. Labeled RNA was added to Affymetrix hybridization cocktails, heated at 99ºC for 5 min and hybridized for 16 h at 48ºC to MicroRNA 4.0 GeneChips (Affymetrix Inc., Santa Clara CA) using the GeneChip Hybridization oven 645. The microarrays were then washed at low (6X SSPE) and high (100mM MES, 0.1M NaCl) stringency and stained with streptavidin-phycoerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycoerythrin stain. A GeneChip 3000 7G scanner was used to collect fluorescence signal. Affymetrix Command Console and Expression Console were used to quantitate expression levels for targeted microRNAs, default values provided by Affymetrix were applied to all analysis parameters.

Antibodies

Anti-HLA-A, -B, and –C antibodies (Santa Cruz) were utilized for NanoSight fluorescent staining and analysis of human islet exosomes purified from islet cultures and for xenoislet recipient mouse plasma analysis. Antibodies to human insulin, glucagon, and somatostatin, CD3, CD4, CD8, CD19, TSG101, aquaporin 2, podocalyxin-1, β-actin, and to mouse MHC I and isotype controls (anti-goat, anti-rabbit, anti-mouse, goat IgG, rabbit IgG, and mouse IgG) were purchased from Santa Cruz Biotechnologies, Inc. Anti-human FXYD2 antibody was purchased from Abnova (Taipei city, Taiwan). Anti-goat, anti-rabbit, and anti-mouse conjugated quantum dot (605 nm) were purchased from Life Technologies (Carlsbad, CA). Unconjugated HLA allele specific antibodies, mouse anti- HLA-A2, -HLA-B27, -HLA-B13, -HLA-B8, were purchased from One Lambda (Canoga Park, CA).

Antibody	**Catalog Number**	**Company**
Aquaporin-2 AQP2 (N-20)	Sc-9889	Santa Cruz
β-Actin monoclonal	66009-1-Ig	Protein Tech
CD3-ε (UCH-T1)	Sc-1179	Santa Cruz
CD63 (H-193)	Sc-15363	Santa Cruz
Cytochrome-C (C-20)	Sc-8385	Santa Cruz
Flotillin-1 polyclonal	15571-1-AP	Protein Tech
FXYD2 Antibody (1C3-B3)	H00000486-M01	Novus Biologicals
Glucagon (FL-180)	13091	Santa Cruz
HLA-A (A-18)	Sc-23446	Santa Cruz
HLA-B (N-20)	Sc-19440	Santa Cruz
HLA-C (Q-18)	Sc-19438	Santa Cruz
Insulin (H-86)	Sc-9168	Santa Cruz
Podocalyxin-like 1 (B-11)	Sc-393716	Santa Cruz
Somatostatin (G-10)	Sc-55565	Santa Cruz
TSG101 (C-2)	Sc-7964	Santa Cruz
HLA-A2 IgM	BIH0648	One Lambda
HLA-A2 IgG	0791HA	One Lambda
HLA-B13 IgM	BIH0261	One Lambda
HLA-B13 IgG	0044HA	One Lambda
HLA-B8 IgG	BIH0536A	One Lambda
HLA-B27 IgG	BIH1453	One Lambda