SUPPLEMENTARY DATA

Supplementary Table 1. Proteins identified by LC-MS/MS analysis in EV preparations from rat pancreatic islets, INS-1E cells and human pancreatic islets

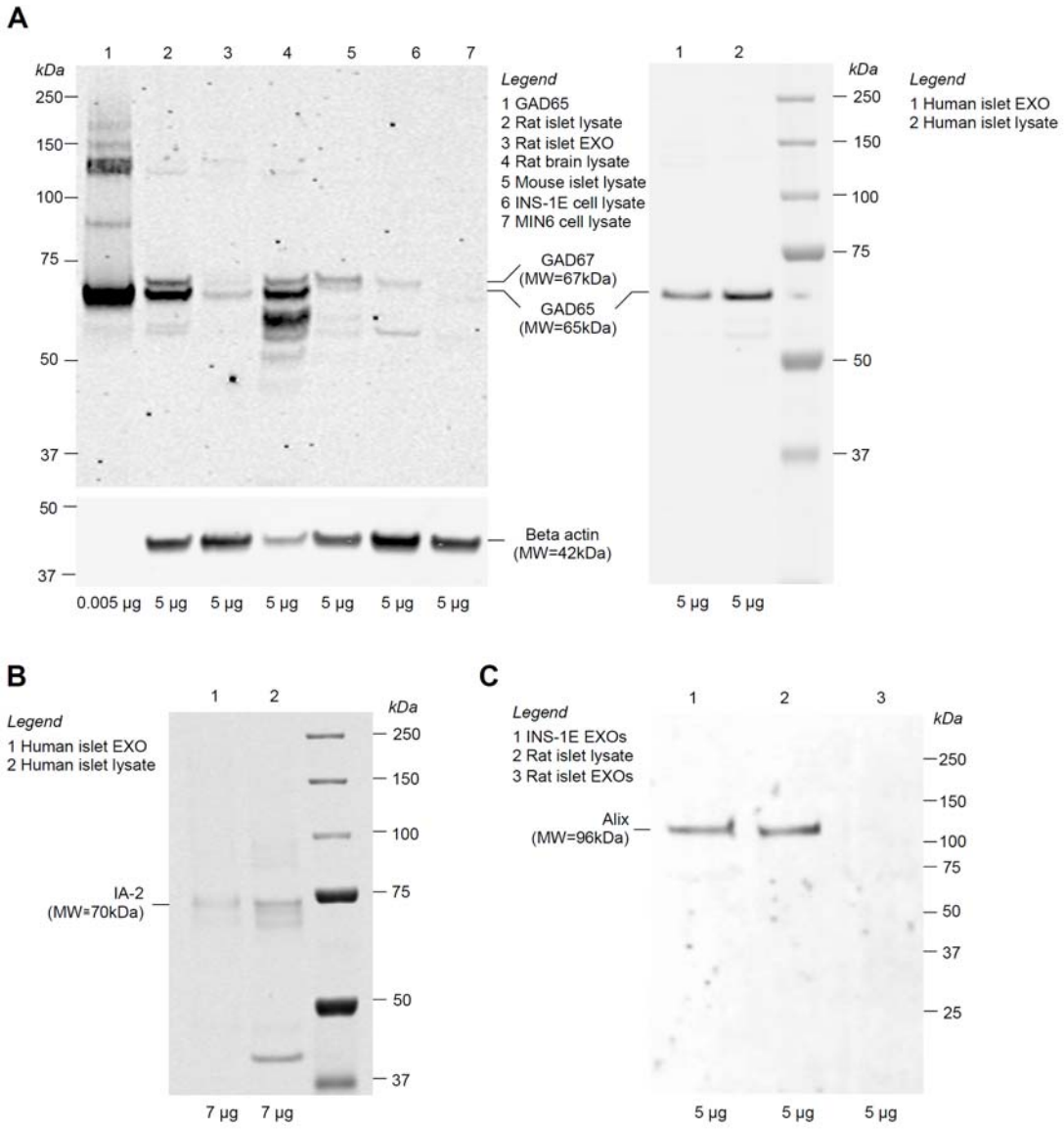
The right column shows references for identification of each protein in exosomal preparations from other cell types.

Pathway	Protein	Uniprot Accession Number	Matched Unique Peptides		Uniprot Accession Number	Matched Unique Peptides	Reference
			Rat islets	INS-1E		Human islets	
Cytoskeleton associated	Beta tubulin	P69897	26	22	P68371	20	(1)
	Alpha Tubulin	Q6P9V9	27	16	P68363	18	(2)
	Actin	P63259	44	24	P63261	30	(3)
	Moesin	F1LP60	19	1	P26038	17	(3)
	Ezrin	P31977	3	0	P15311	35	(4)
	Profilin1	P62963	5	3	P07737	6	(5)
	Cofilin1	P45592	5	8	P23528	9	(1)
Signaling	14-3-3 protein zeta/delta	P63102	26	12	P63104	12	(1)
	14-3-3 protein epsilon	P62260	12	8	P62258	8	(3)
	Elongation factor 1-alpha 1	P62630	16	14	P68104	18	(6)
Membrane trafficking	Annexin 2	Q07936	11	0	P07355	25	(6)
	Annexin 6	D4ABR6	15	0	P08133	35	(7)
	Rab1	E9PU16	5	3	P62820	3	(8)
	Rab11a	P62494	10	2	P62491	4	(9)
	Rab27a	P23640	2	0	H3BN55	3	(10)
	Rab27b	Q99P74	5	0	O00194	0	(10)
	Rab35	Q5U316	2	1	F5H157	2	(11)
	Flotillin1	Q9Z1E1	2	0	O75955	0	(12)
	Clathrin heavy chain	F1M779	67	89	Q00610	52	(2)
Tetraspanins	CD9	P40241	1	0	A6NNI4	2	(13)
	CD81	Q6P9V1	3	5	A6NMH8	3	(6)
Late endosome/ Lysosome	LAMP1	P14562	1	1	P11279	4	(13)
Enzymes	GAPDH	P04797	13	16	P04406	22	(2)
	Pyruvate kinase M2 isoform	P11980-2	18	17	P14618	29	(2)
Heat shock response	Hsc70	P63018	24	37	E9PKE3	19	(1)
	Hsp70	Q07439	12	2	P08107	15	(4)
	Hsp90alpha	P82995	3	4	P07900	18	(3)
	Hsp90beta	P63018	7	14	P08238	13	(3)
Beta cell islet antigen	Insulin1	P01322	7	12	P01308	20	
	Insulin2	P01323	4	11			

SUPPLEMENTARY DATA

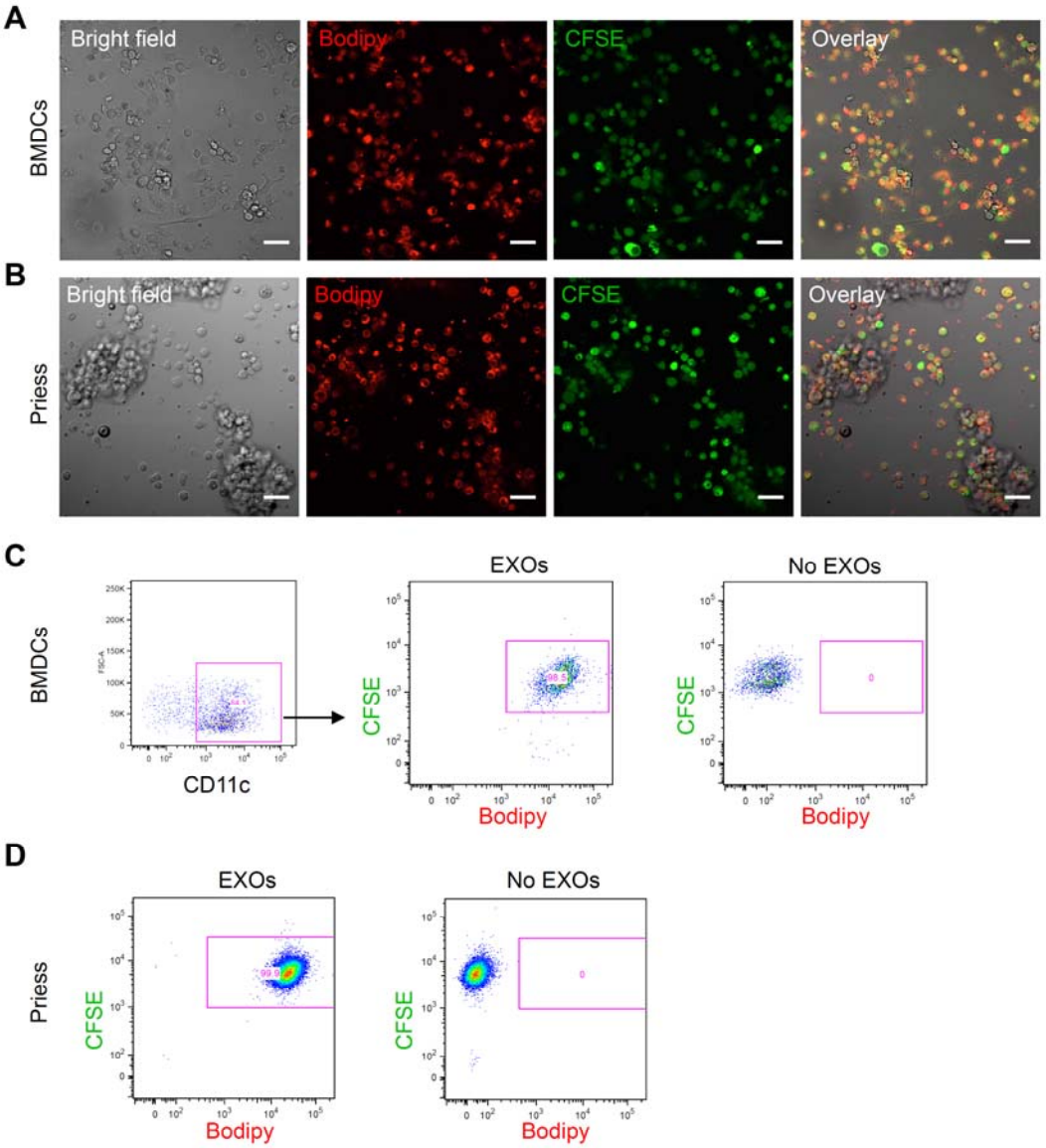
Supplementary Figure 1. Analyses of protein expression in exosomes isolated from primary islets or insulinoma cells

(**A**) Uncropped scan related to the cropped scan of a Western blot showing GAD65/GAD67 in rat and human islets and exosomes in Figure 1. The uncropped Western blot shows rat, mouse and human islet proteins, rat and human exosome proteins and rat and mouse insulinoma protein immunostained using the GAD1701 antiserum that recognizes both GAD65 and GAD67. Rat islets express both GAD65 and the non-autoantigenic isoform GAD67 (left panel), while human islets only express the GAD65 isoform of GAD (right panel). Mouse islets and rat INS-1E cells only express GAD67, while mouse MIN6 cells express neither GAD isoform (left panel). Human recombinant GAD65, rat brain lysate and rat islet lysate were used as positive controls. (**B**) Uncropped scan related to the cropped scan of a Western blot showing human IA-2 in human islets and exosomes in Figure 1E. The Western blot of human islet and exosome proteins was immunostained with the 76F-B4 mouse monoclonal antibody to IA-2. (**C**) Western blot of rat islet exosome proteins, rat islet proteins, and rat insulinoma cell line INS-1E exosome proteins immunostained with a monoclonal antibody to the exosomal marker Alix. (**A-C**) Data are representative of 2-5 independent experiments.



SUPPLEMENTARY DATA

Supplementary Figure 2. Uptake of islet exosomes by BMDCs and Priess B cells
Pancreatic islet derived-EVs were labeled with Bodipy-Ceramide and added at the concentration of 25 µg/ml to 100,000 CFSE-labeled $DR4^{+/+}$ BMDCs (**A, C**) or $DR4^{+/+}$ human Priess B cells (**B**, **D**). After five hours of incubation, cells were analyzed by live cell imaging (**A, B**) and flow cytometry (**C, D**). Confocal acquisitions of CFSE (green), Bodipy-Ceramide (red) and bright field channels show that both DCs (**A**) and B cells (**B**) are positive for Bodipy-Ceramide. In addition, a very high percentage (around 99%) of BMDCs cells (**C**) and B cells (**D**) are Bodipy-Ceramide-positive by flow cytometry. Analyses of BMDCs were performed on the cell population positive for the CD11c antigen expressed by mature dendritic cells. Scale bars: 30 µm.



SUPPLEMENTARY DATA

Mice

$DR4^{+/+}$NOD mice were obtained from Dr. Grete Sønderstrup, Stanford University. These mice are homozygous for the human T1D MHC-class II susceptibility haplotype DRA1*0101, DRB1*0401 (hereafter referred to as DR4) and express DR4 antigens as well as human CD4 in lymphoid cells, and human proinsulin in pancreatic β cells. The mice are negative for expression of murine MHC-class II IA and IE antigens (14). They do not develop T1D.

Human islet donors

Human islets, obtained through the European Consortium for Islet Transplantation (ECIT) Islets for Basic Research Program, were from six non-diabetic donors, four males and two females, age 36-59 years with a body mass index of 20-29 kg/m^2. The causes of death were stroke (3 donors), cerebral bleeding (1 donor) and cardiac arrest (1 donor). At reception, islets were purified to 95-100% by gravity sedimentation through complete medium and by handpicking under a stereo microscope. Humans islets were cultured in Connaught Medical Research Laboratories 1066 (CMRL) medium with 2 mM L-Glutamine, 25 mM HEPES, 1% P/S at $25^{o}C$ in 5% CO_2.

Mass spectrometry

Preparation of protein samples for MS was carried out as previously described (15). In brief, EV samples were digested in solution and peptides were desalted using StageTips and dried in a vacuum concentrator. For LC-MS/MS analysis, resuspended peptides were separated by reverse phase chromatography on a Dionex Ultimate 3000 RSLC nano UPLC system connected in-line with an Orbitrap Elite (ThermoFisher Scientific). Database search was performed using Mascot 2.5 (Matrix Science) and SEQUEST in Proteome Discoverer v.1.4. against a human or rat Uniprot protein database. Data were further processed and inspected in Scaffold™ 4.2.1 (Proteome Software). Normalized quantitative values were utilized to compare the protein profile in exosomes from three different preparations of untreated and cytokine-treated rat islets. The normalization scheme in Scaffold adjusts the sum of the selected quantitative value for all proteins in the list within each MS sample to a common value: the average of the sums of all MS samples present in the experiment. This is achieved by applying a scaling factor for each sample to each protein or protein group adjusting in this way the selected value to a normalized "Quantitative Value". Normalized quantitative values were utilized to compare the protein profile in exosomes from untreated and cytokine-treated rat islets. For proteomic detection of GAD65 and IA-2, exosomal proteins were separated by SDS-PAGE (Invitrogen). Proteins were eluted from gel slices corresponding to the relative mobility of GAD65 and IA-2 and subjected to LC-MS/MS analyses (15).

SDS-PAGE and Western Blot analysis

Cell lysates were prepared by extraction of 200 islets or 2 x 10^6 INS-1E or MIN6 cells in 200 µl of RIPA buffer (Sigma). Brain extracts from P5 rats were obtained by collagenase D (Roche) digestion on a 40 µm cell strainer. After centrifugation at 600xg for 5 minutes at 4°C, the pellet was extracted in RIPA buffer. The BCA protein assay kit (ThermoFisher Scientific) was used to measure the protein concentration of cell extracts. A total of 5-15 µg protein per lane was analysed SDS-PAGE and Western blotting (WB), as described (16). Recombinant human GAD65 used for standard was from FIRS Laboratories RSR. Primary antibodies were: GAD1701 (rabbit anti-GAD65/GAD67) (17), 1:5000; rabbit anti-flotillin1 (donated by G. Van der Goot), 1:2000; mouse anti-CD9 antibody (Santa Cruz, sc-13118), 1:100; 76F-B4 (mouse anti-IA-2, donated by E. Bonifacio) (18), 1:20; mouse anti-beta actin (Sigma, A1978), 1:2000; chicken anti-Calreticulin (Abcam, ab14234), 1:1000; rabbit anti-Gp96 (Abcam, ab13509), 1:1000; mouse anti-PDI (BD Transduction Laboratories, 610947), 1:500; and mouse anti-Alix (Cell Signaling, 2171), 1:1000.

SUPPLEMENTARY DATA

REFERENCES

1. Théry C, Boussac M, Véron P, Ricciardi-Castagnoli P, Raposo G, Garin J, et al. Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immunol 2001;166(12):7309–18
2. Wubbolts R, Leckie RS, Veenhuizen PTM, Schwarzmann G, Möbius W, Hoernschemeyer J, et al. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 2003;278(13):10963–72
3. Carayon K, Chaoui K, Ronzier E, Lazar I, Bertrand-Michel J, Roques V, et al. Proteolipidic composition of exosomes changes during reticulocyte maturation. J Biol Chem 2011;286(39):34426–39
4. Hegmans JPJJ, Bard MPL, Hemmes A, Luider TM, Kleijmeer MJ, Prins J-B, et al. Proteomic analysis of exosomes secreted by human mesothelioma cells. Am J Pathol 2004;164(5):1807–15
5. Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, et al. Cells release prions in association with exosomes. Proc Natl Acad Sci U S A 2004;101(26):9683–8
6. Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gil D, Matthiesen R, Valle M, et al. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. J Proteome Res 2008;7(12):5157–66
7. Pisitkun T, Shen R-F, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 2004;101(36):13368–73
8. Hassani K, Olivier M. Immunomodulatory Impact of Leishmania-Induced Macrophage Exosomes: A Comparative Proteomic and Functional Analysis. Milon G, editor. PLoS Negl Trop Dis 2013;7(5):e2185
9. Savina A, Vidal M, Colombo MI. The exosome pathway in K562 cells is regulated by Rab11. J Cell Sci 2002;115(Pt 12):2505–15
10. Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 2010;12(1):19–30; sup pp 1–13
11. Hsu C, Morohashi Y, Yoshimura S-I, Manrique-Hoyos N, Jung S, Lauterbach MA, et al. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. J Cell Biol 2010;189(2):223–32
12. Strauss K, Goebel C, Runz H, Möbius W, Weiss S, Feussner I, et al. Exosome secretion ameliorates lysosomal storage of cholesterol in Niemann-Pick type C disease. J Biol Chem 2010;285(34):26279–88
13. Nazarenko I, Rana S, Baumann A, McAlear J, Hellwig A, Trendelenburg M, et al. Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. Cancer Res 2010;70(4):1668–78
14. Congia M, Patel S, Cope AP, De Virgiliis S, Sønderstrup G. T cell epitopes of insulin defined in HLA-DR4 transgenic mice are derived from preproinsulin and proinsulin. Proc Natl Acad Sci U S A. 1998;95:3833–8
15. Chopra T, Hamelin R, Armand F, Chiappe D, Moniatte M, McKinney JD. Quantitative Mass Spectrometry Reveals Plasticity of Metabolic Networks in Mycobacterium smegmatis. Mol Cell Proteomics 2014;13(11):3014–28
16. Phelps EA, Cianciaruso C, Michael IP, Pasquier M, Kanaani J, Nano R, et al. Aberrant Accumulation of the Diabetes Autoantigen GAD65 in Golgi Membranes in Conditions of ER Stress and Autoimmunity. Diabetes 2016;65(9):2686–99
17. Kim J, Richter W, Aanstoot HJ, Shi Y, Fu Q, Rajotte R, et al. Differential expression of GAD65 and GAD67 in human, rat, and mouse pancreatic islets. Diabetes 1993;42(12):1799–808
18. Piquer S, Valera L, Lampasona V, Jardin-Watelet B, Roche S, Granier C, et al. Monoclonal antibody 76F distinguishes IA-2 from IA-2β and overlaps an autoantibody epitope. J Autoimmun. 2006;26(3):215–22

SUPPLEMENTARY DATA

Cianciaruso et al.,
Primary human and rat beta cells release the intracellular autoantigens GAD65, IA-2 and proinsulin in exosomes together with cytokine-induced enhancers of immunity

Justification for Online Supplemental Material:

Supplementary Table 1. shows a list of proteins identified by LC-MS/MS in EV preparations from primary human and rat islets and from the rat insulinoma cell line INS-1E. Information about the matched unique peptides identified and uniprot accession number are provided for each protein. References in which the same proteins were identified in exosomes from other cell types are listed in the last column. This information is non-essential for comprehension of the main text but relevant for showing the main categories of proteins in islet-EV preparations and that they are shared with exosomal proteins isolated from other cells.

Supplementary Figure 1. shows the uncropped scans of Western blots of GAD65 and IA-2 reported in Figure 1E and 1F respectively. It also shows the lack of detection of the exosomal protein Alix in rat islet exosomes. Part A, left panel is important because it reveals the presence of GAD65 in rat islet exosomes and rat islet and brain lysates, while it is absent in mouse islet, rat insulinoma INS-1E cell, and mouse MIN6 insulinoma cell lysates. Thus, it emphasizes the importance of selecting primary human or rat islet cells for studies on the GAD65 protein. Part A, right panel is important because is reveals the specificity of detection of GAD65 in human islet exosomes. Part B is important because it reveals the specificity of detection of IA-2 in human islet-exosomes. Part C is important because it reveals that while Alix is present in INS-1E cell exosomes, it is not detected in rat islet exosomes providing evidence that primary beta cell and insulinoma cell exosomes diverge in protein composition.

Supplementary Figure 2. provides data on live cell imaging and flow cytometry analyses of uptake of Bodipy-labeled islet-exosomes by BMDCs and by Priess cells. This information expands the data shown in Figure 3A and provides evidence for uptake of islet exosomes by not only DR4-positive BMDCs but also by the DR4-positive human B cell line Priess.

Supplementary Data, provide details of $DR4^{+/+}$mice, human islet donors, mass spectrometry, SDS-PAGE and WB analysis.