Supplementary Information

Extracellular Vesicles from Human Pancreatic Islets Suppress Human Islet Amyloid Polypeptide (IAPP) Amyloid Formation

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Content

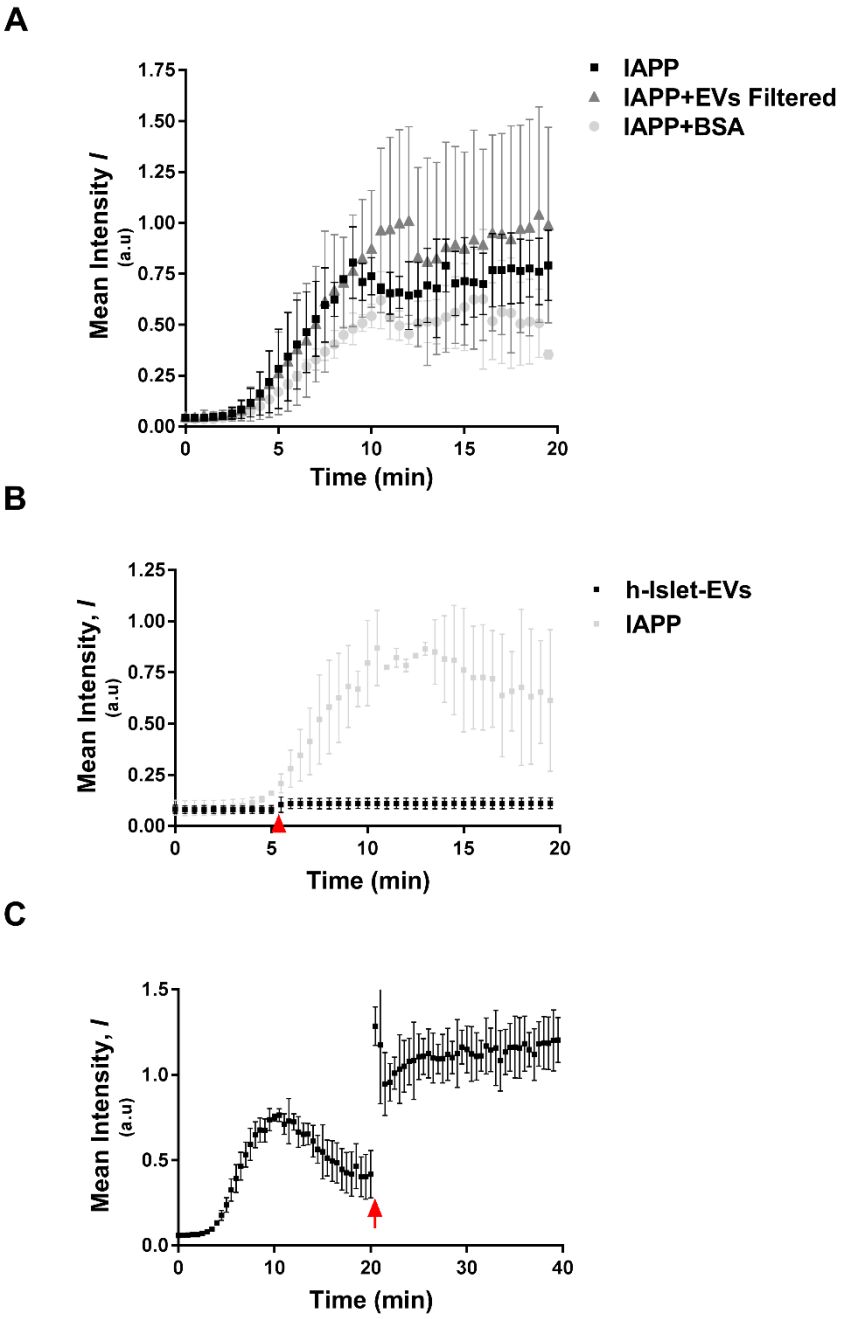

Figure S1. Control aggregation experiments with EV media, BSA and EVs. A) ThT fluorescence of IAPP amyloid formation in the presence of filtered EV solution (removing the EVs but retaining everything in solution) and BSA at 25 µg/mL. B) ThT fluorescence of human islet EVs incubated without IAPP (added at red arrow) and IAPP alone for comparison. C) ThT fluorescence of IAPP amyloid formation without EVs for 20 min followed by EV addition at red arrow (i.e., after amyloid formation). There is an instant jump in the ThT signal, likely related to solubilization of precipitated amyloids, but then the signal remains rather constant. This indicates that EVs cannot dissolve pre-formed IAPP amyloids. For all, data is displayed as mean±SD.

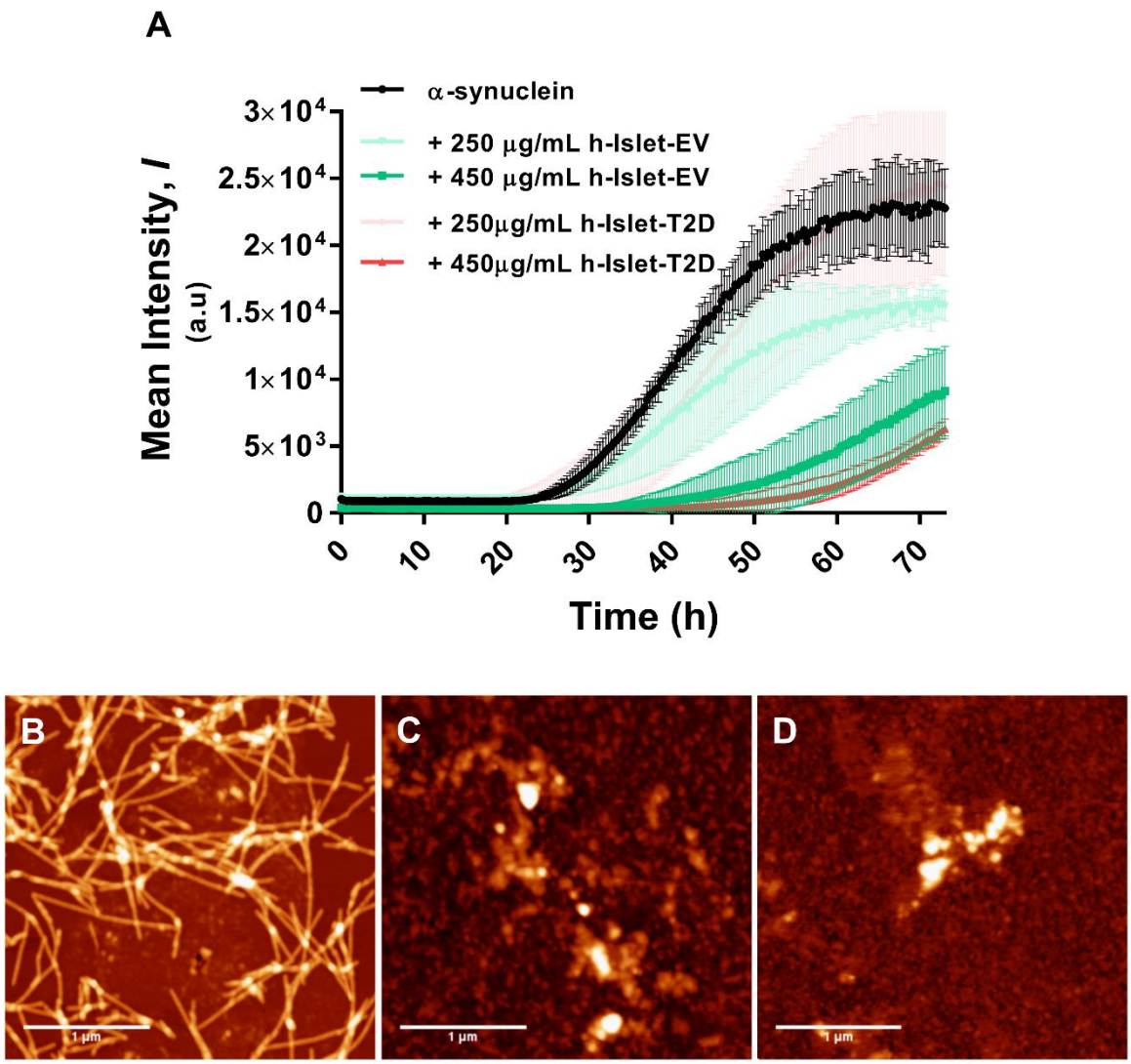

Figure S2. Aggregation of α-synuclein in the presence of pancreatic EVs. A) ThT fluorescence versus time (h), using EV concentrations of 250 μg/ml and 450 μg/ml. No significant differences are detected upon EV additions at 250 μg/ml. When the EV concentration is raised to 450 μg/ml (giving the same EV-to-protein ratio as in the IAPP experiments), retardation of amyloid formation is observed for both healthy and T2D pancreatic EVs. Data is displayed as mean ± SD. AFM images of samples after aggregation experiments (i.e., at 80 h) for α-synuclein alone (B), α-synuclein with 450 μg/ml h-islet-EVs (C) and α-synuclein with 450 μg/ml h-T2D-EVs (D). In C and D, the visible particles have heights of approx 30-50 nm which imply that they are exosomes but they could also be non-fibrillar protein aggregates.

A

	SE	TAG	CH	PE	PC	SM	SUM (mg/L)	
h-Islet-EV	42,3±9,2	23,1±4,3	12,4±2,5	25,8±4,3	24,3±4,4	6,5±1,1	134,4±11,8	mean±SD
	100	**55**	**29**	**61**	**57**	**15**	**%**	
h-T2D-EV	34,2±0,2	19,7±0,6	8,8±0,8	21,7±0,2	18,7±0,2	4,5±0,1	107,6±10,5	mean±SD
	100	**58**	**26**	**64**	**55**	**13**	**%**	
h-Ctr-EV	30,6±1,2	15,3±0,5	5,3±0,4	13,4±0,8	9,2±1,0	2,8±0,2	76,6±9,9	mean±SD
	100	**50**	**17**	**44**	**30**	**9**	**%**	

B

	SE	TAG	CH	PE	PC	SM
h-Islet-EV vs. h-T2D-EV	Ns	Ns	Ns	Ns	Ns	Ns
h-Islet-EV vs. h-Ctr-EV	***	Ns	Ns	Ns	**	Ns
h-T2D-EV vs. h-Ctr-EV	**	Ns	Ns	Ns	Ns	Ns

Figure S3. **EV lipid identification and quantification.** A) Quantification of six classes of lipids identified in the different EV samples. Concentrations detected are given in mg/ml of each lipid as well as relative amount of each lipid with the most abundant lipid (SE in each case) set to 100 %. B) 2 way-ANOVA statistical analysis of data in A. Samples from one donor per condition were measured in triplicate. *Represents statistical significance, Ns represents No significance. Steryl ester (SE), triacylglycerol (TAG), cholesterol (CH), phosphatidylethanolamine (PE), sphingomyelin (SM), and phosphatidylcholine (PC).

Table S1. Information of the h-Islets donors from which samples were used for EVs isolation.

#Batch	Diabetic	Gender	Age	BMI	COD - cause of death
1	No	Male	29	22,4	Head trauma
2	No	Female	69	22,7	Stroke
3	No	Male	23	24,8	Anoxia event
4	No	Male	38	23,2	Stroke
			40,3	**23,3**	
1	Yes	Female	55	29,8	Anoxia event
2	Yes	Female	42	27,6	Motor vehicle trauma
3	Yes	Female	56	29,5	Stroke
			51,0	**29,0**	

Table S2. P-value description.

P-value	Wording	Symbol summary
< 0.0001	Extremely significant	****
0.0001 to 0.001	Extremely significant	***
0.001 to 0.01	Very significant	**
0.01 to 0.05	Significant	*
≥ 0.05	Not significant	