SUPPLEMENTARY DATA

Supplementary Table 1. Human islet donors used in each figure.

Figure Number	ND donors	T2D donors
Figure 1A	R022, R030, R047, R058, H1557, H1567, H1570, H1647, H1657, H1693, H1695	
Figure 1B	R073, R075, R157, H744, H1759	R155
Figures 1C and 1D	R058, R062, R066, H1664	
Figures 1E and 1F	R058, R069, R072, R077, H1759	
Figure 2E	H1987, H1988, H1991	
Figure 8A		R152, R155
Figure 8B		R152, R154, R155
Figure 8C	R022, R030, R047, R058, H1557, H1567, H1570, H1647, H1657, H1693, H1695	R023, R024, R031, R054, R057
Figure 8D and 8F	R136, R145, R159	R152, R154, R155
Figure 8E and 8F		R057, R059, R078, R080, R083
Figure 8G, 8H and 8I	R200, R202	R198, R201
Figure 8J and 8K		R078
Figure 8L		R063, R078, R080
Suppl. Figure 1	R060, R061, H1743, H1744, H1745	
Suppl. Figure 5B and 5C	R179, R180, R181, H1991, H1992, H1997	
Suppl. Figure 7A, 7B and 7C	R072, R074, R094	R063, R078, R080

ND – no diabetes; T2D – type 2 diabetes

SUPPLEMENTARY DATA

Supplementary Table 2. Characteristics of human islet donors with no diabetes. Preparations were processed by the Alberta Diabetes Institute Isletcore (Donor Numbers with 'R') or the Clinical Islet Laboratory at the University of Alberta (Donor Numbers with 'H').

Donor Number	Age (years)	Sex	Donor Type	Cause of Death (if known)	BMI	HbA1c	CIT (hours)	Isolation Purity (%)
R022	48	F	NDD		18.0		21.5	30
R030	80	F	NDD	SAH/CVA/SIH	21.9		12.0	90
R047	56	F	NDD	SAH/CVA/SIH	32.0		17.0	50
R058	79	F	NDD	SAH/CVA/SIH	23.8	5.8	16.5	75
R060	54	F	NDD	Cardiac Arrest	21.1	5.8	17	90
R061	63	M	NDD	SAH/CVA/SIH	37.6	5.7	11	80
R062	41	M	NDD	SAH/CVA/SIH	19.7	5.2	9.3	90
R066	44	M	NDD	Anoxia	32.2		12.0	80
R069	74	F	NDD		25.1		23.3	<10
R072	54	F	NDD	SAH/CVA/SIH	30.6	6.2	10.1	90
R073	74	F	NDD	SAH/CVA/SIH	28.3	5.4	13.0	90
R074	55	M	DCD	Head Injury/Trauma	26.4	5.5	15.0	10
R075	27	M	NDD	Fentanyl overdose	26.2	5.4	15.3	50
R077	54	F	NDD	Head Injury/Trauma	24.7	5.2	15.0	90
R094	46	F	NDD	Head Injury/Trauma	28.1	5.8	23.8	70
R136	60	F	NDD	SAH/CVA/SIH	36.3	6.2	18.0	50
R145	55	F	NDD	SAH/CVA/SIH	24.1	5.5	13.5	80
R159	60	M	NDD	SAH/CVA/SIH	30.4	5.5	2.0	70
R179	61	M	NDD	Cardiac Arrest	25.1	5.7	7.3	80
R180	68	F	NDD	SAH/CVA/SIH	21.7	5.9	23.3	95
R181	42	F	DCD	Head Injury/Trauma	29.4	6.0	19.3	95
R200	65	M	NDD	SAH/CVA/SIH	27.1	5.1	17.5	95
R202	68	F	NDD	Head Injury/Trauma	25.2	5.7	16.5	90
H1557	27	F	NDD	Anoxia	21.6	5.8	5.5	55
H1567	52	F	NDD	SAH/CVA/SIH	21.8	6.0	9.3	40
H1570	45	M	NDD	Encephalitis	25.7	6.3	4.8	53
H1647	62	F	NDD	SAH/CVA/SIH	25.9	5.3	7.5	90
H1657	49	F	NDD	SAH/CVA/SIH	29.5	6.3	4.8	65
H1664	60	M	NDD	Head Injury/Trauma	30.4	5.9	11.3	80
H1693	51	F	NDD	SAH/CVA/SIH	29.9	6.2	13.5	40
H1695	47	M	NDD	Head Injury/Trauma	21.4	5.8	4.3	39
H1743	73	F	NDD	SAH/CVA/SIH	21.7	5.4	10.8	43
H1744	56	F	NDD	SAH/CVA/SIH	16.7	5.9	14.3	30
H1745	47	M	NDD	Head Injury/Trauma	25.9	6.4	8.8	25
H1759	48	M	NDD	SAH/CVA/SIH	26.0	5.2	7.3	45
H1987	36	F	NDD	Anoxia	28.8	5.8	10.8	40
H1988	61	M	NDD	SAH/CVA/SIH	29.0	6.0	11.0	40
H1991	54	F	NDD	SAH/CVA/SIH	22.2	6.1	11.8	30
H1992	63	F	NDD	Anoxia	21.5	5.3	10.8	40
H1997	75	M	NDD	Anoxia	26.0	6.0	11.5	48
Average	55.9				26.0	5.8	12.7	61.3
SEM	2.0				0.7	0.1	0.8	4.1

NDD – neurological determination of death; DCD – donation after cardio-circulatory death; SAH – subarachnoid hemorrhage; CVA – cerebrovascular accident (stroke); SIH – spontaneous intracranial hemorrhage

SUPPLEMENTARY DATA

Supplementary Table 3. Characteristics of human islet donors with type 2 diabetes (T2D). All preparations were processed by the Alberta Diabetes Institute Isletcore.

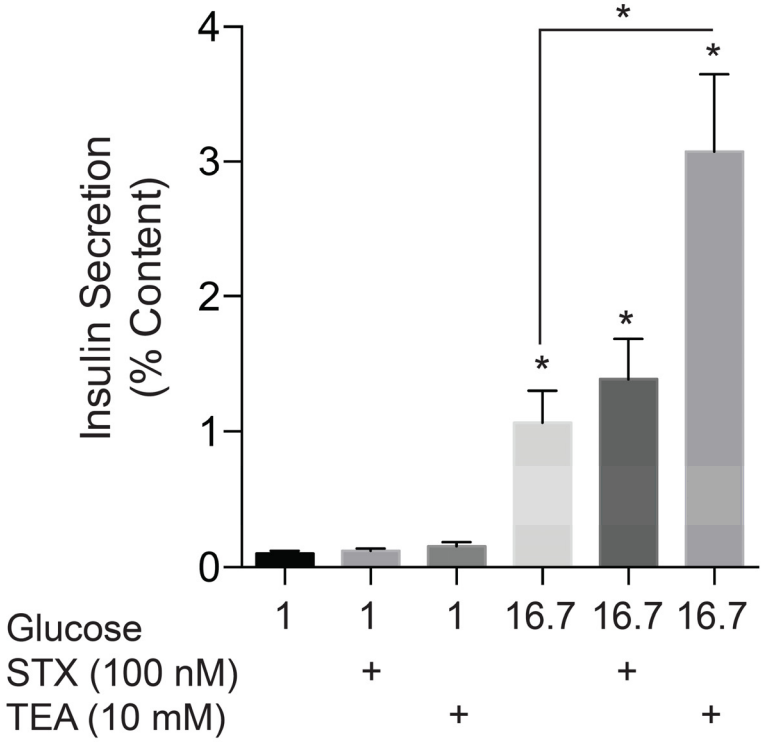
Donor Number	Age (years)	Sex	Donor Type	Cause of Death (if known)	BMI	HbA1c	Years T2D (if known)	T2D Medications	CIT (hours)	Isolation Purity (%)
R023	65	M	NDD	Anoxia	29.3	8.3		unknown	44.5	30
R024	52	F	NDD		29.0		5	unknown	23.0	45
R031	54	M	NDD	SAH/CVA/SIH	30.0	7.2		unknown	13.0	50
R054	73	F	NDD		22.4	6.1	5	unknown		60
R057	53	F	NDD	SAH/CVA/SIH	35.5	10.3	20	metformin	15.0	10
R059	68	F	NDD		28.1			unknown	13.1	70
R063	57	F	NDD	Anoxia	23.0	6.1	2	unknown	12.0	95
R078	47	F	NDD	Cardiac Arrest	35.3	5.9	6	unknown	11.0	5
R080	57	M	NDD	Head Injury/Trauma	28.3	5.8	5	metformin	10.0	75
R083	71	F	NDD	Anoxia	27.5	6.6	20	unknown	11.5	75
R152	54	F	DCD	SAH/CVA/SIH	42.6	8.3	4	metformin, gliclazide	14.5	5
R154	57	F	NDD	SAH/CVA/SIH	40.8	7.2	4	metformin	6.0	20
R155	56	M	DCD	Cardiac Arrest	34.0	6.3	2	gliclazide	18.5	50
R198	47	F	DCD		29.7	5.4	10	unknown	12.5	50
R201	69	M	NDD	Head Injury/Trauma	32.2	6.7		unknown	18.8	90
Average	58.7				31.2	6.9	7.5		16.0	48.7
SEM	2.1				1.5	0.4	2.0		2.5	7.7

NDD – neurological determination of death; DCD – donation after cardio-circulatory death; SAH – subarachnoid hemorrhage; CVA cerebrovascular accident (stroke); SIH – spontaneous intracranial hemorrhage

SUPPLEMENTARY DATA

Supplementary Figure 1. Pharmacologic Kv2 inhibition does not enhance insulin secretion from human islets.

Insulin secretion was measured from human islets at 1 or 16.7 mM glucose in the presence or absence of the Kv2.1/2.2 inhibitor stromatoxin (STX, 100 nM), or the non-selective Kv channel inhibitor tetraethylammonium TEA, 10 mM). Data are from 5 separate human islet donors. *p<0.05 compared with the 1 mM glucose condition, or as indicated.



SUPPLEMENTARY DATA

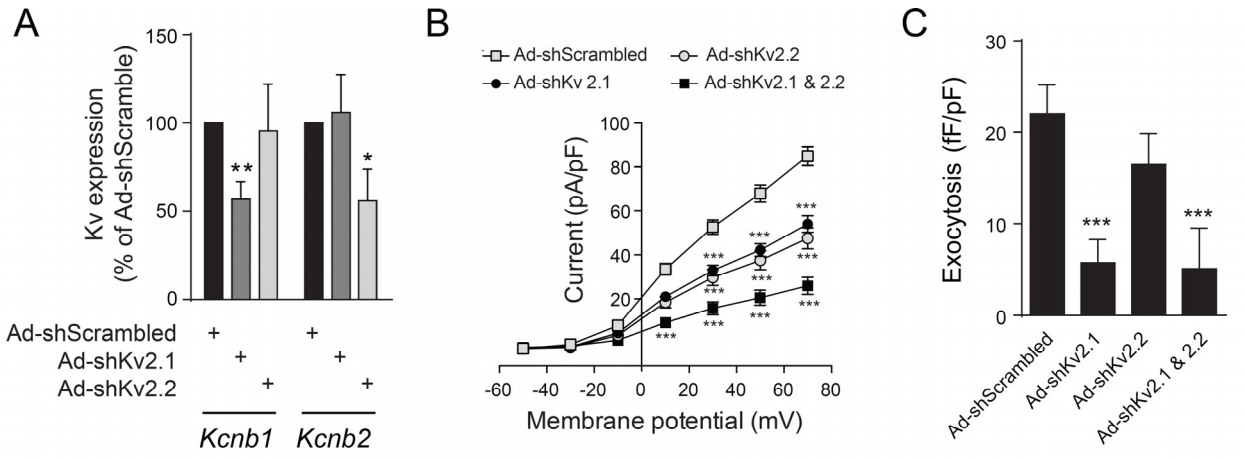
Supplementary Figure 2. Knockdown of the Kv2.1 isoform selectively impairs depolarization-induced exocytosis in INS 832/13 cells.

A) Expression of mRNA for Kv2.1 (*Kcnb1*) or Kv2.2 (*Kcnb2*) in INS 832/13 cells following infection with adenovirus expressing scrambled shRNA (Ad-shScrambled), or targeting Kv2.1 (Ad-shKv2.1) or Kv2.2 (Ad-shKv2.2).
n=3 separate experiments.

B) Current-voltage-relationship of Kv currents recorded by whole-cell voltage-clamp from a holding potential of -70 mV in INS 832/13 cells transduced with adenovirus as indicated. n=27,20,18,19 cells.

C) Cumulative exocytotic responses to a series of 10 membrane depolarizations from -70 to 0 mV by INS 832/13 cells transduced with adenovirus as indicated. n=15,16,11,16 cells.

*-p<0.05, **-p<0.01, and ***-p<0.001 compared with Ad-sh-Scrambled.

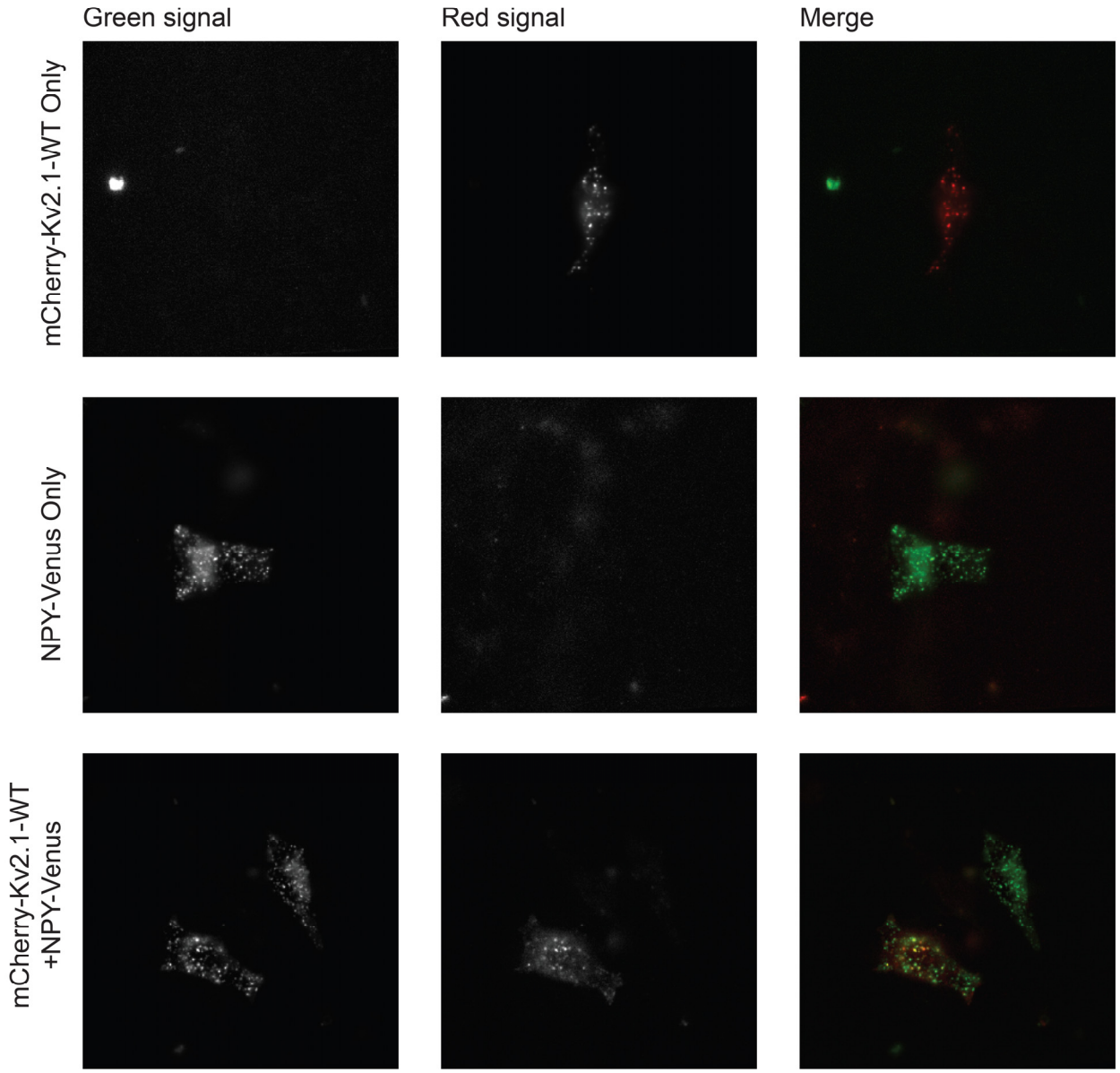


SUPPLEMENTARY DATA

Supplementary Figure 3. Separation of mCherry-Kv2.1-WT and NPY-Venus signals.

INS 832/13 cells were expressing either mCherry-Kv2.1-WT alone (red), NPY-Venus alone (green) or both, and imaged using TIRF microscopy as outlined in the Methods. Bleedthrough between channels was not detected. In the bottom example two cells transfected with NPY-Venus are shown, only one of which is co-expressing mCherry-Kv2.1-WT.

In some of these images (i.e. the negative green - top left -, and negative red - middle) we have increased the brightness relative to other images in order to detect possible bleed-through.



SUPPLEMENTARY DATA

Supplementary Figure 4. Wild-type (wt) and clustering-deficient (ΔC318) Kv2.1 tagged with mCherry mediate identical outward K+ currents, but only the wild-type channel facilitates exocytosis.

A) mCherry-Kv2.1-WT and mCherry-Kv2.1-ΔC318 visualized in HEK 293 and INS 832/13 cells by total internal reflection fluorescence (TIRF) microscopy.

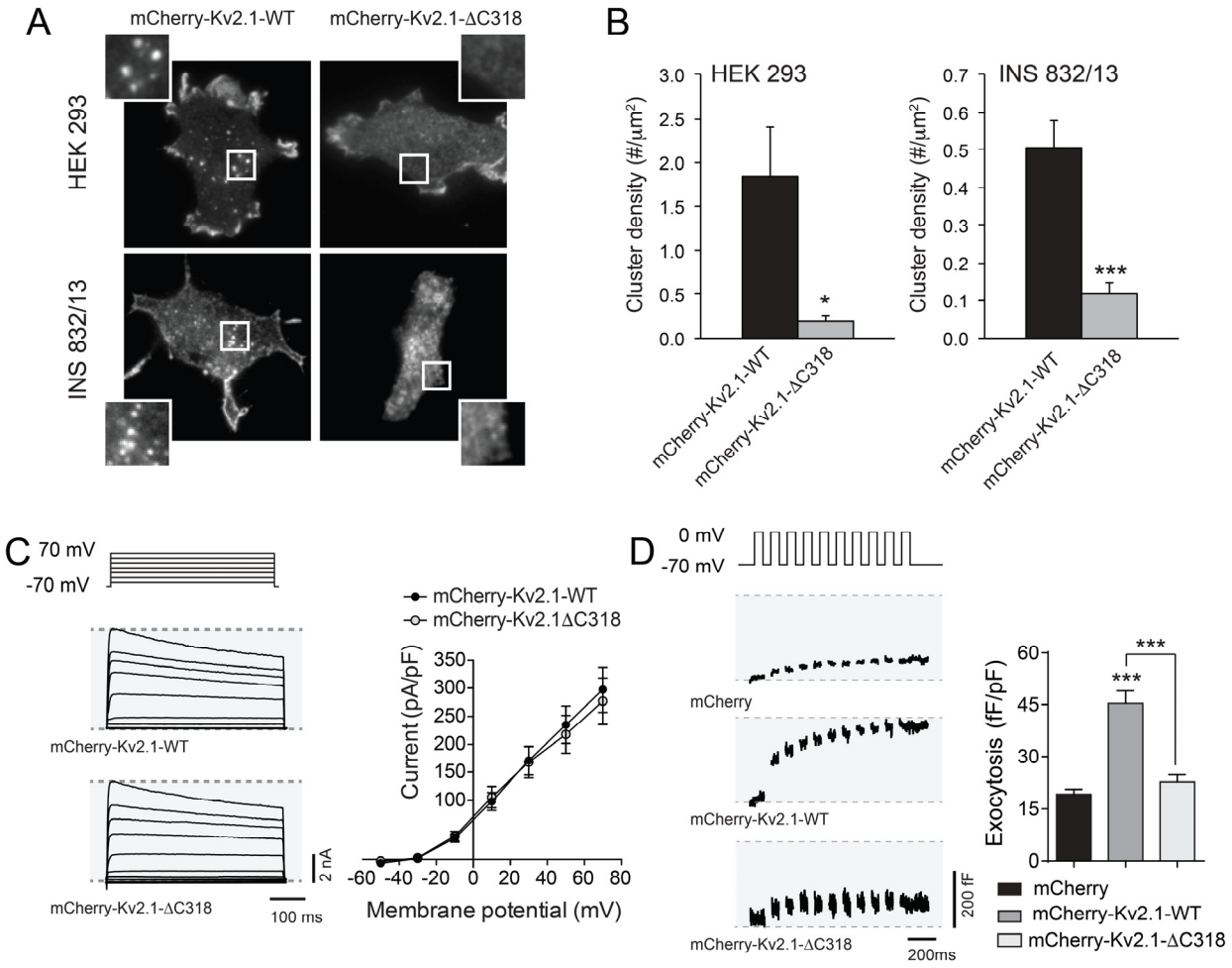
B) The clustering-deficient mutant (ΔC318) forms fewer membrane-localized clusters in both HEK 293 (n=8, 9 cells) and INS 832/13 cells (n=20, 22 cells).

C) Current-voltage relationship of outward K+ currents mediated by mCherry-Kv2.1-WT and mCherry-Kv2.1-ΔC318 expressed in HEK 293 cells. Representative traces (left) and averaged data (right) are shown. n=20, 20 cells.

D) Exocytosis was elicited by a series of 10 depolarizations from -70 to 0 mV in INS 832/13 cells transfected with control plasmid (mCherry), mCherry-Kv2.1-WT, or mCherry-Kv2.1-ΔC318. Representative capacitance traces (left) and averaged cumulative responses (right) are shown. n=29, 31, 32 cells.

*-$p<0.05$ and ***-$p<0.001$ compared with the wild-type or mCherry groups.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 5. Kv2.1-ΔC318 combines retains some ability to cluster by combining with endogenous Kv2.1 in INS 832/13 cells

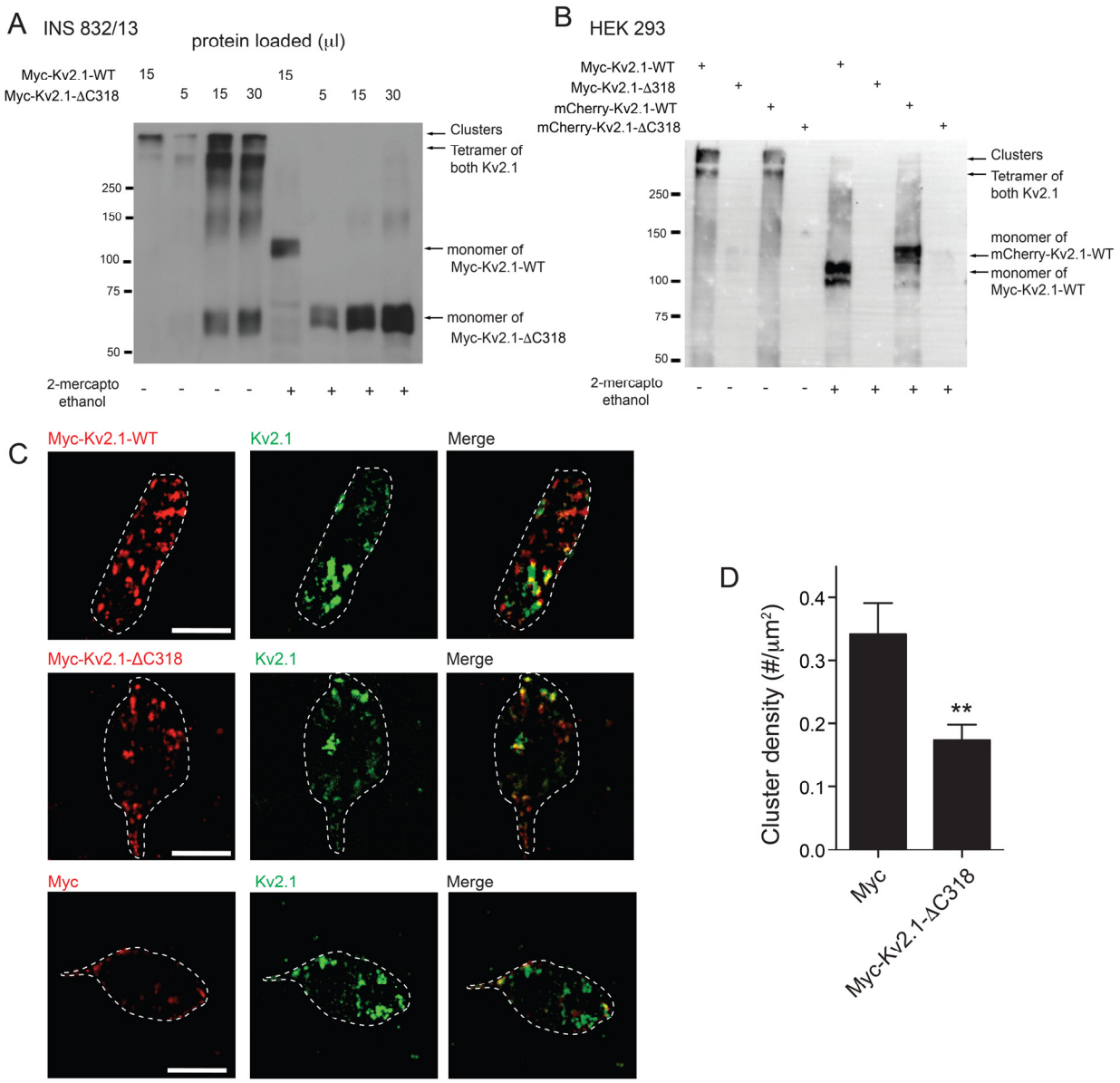
A) Although much of the Myc-Kv2.1-ΔC318 expressed in INS 832/13 cells can be observed as extracellular Kv2.1 channel monomers (i.e. tetrameric single channels) a portion can still form clusters that are evident even as the amount of loaded protein on the gel is reduced. Membrane is blotted with anti-Myc.

B) The anti-Kv2.1 antibody used here is known to recognize a C-terminal epitope that is lacking in the Kv2.1-ΔC318 constructs. We confirm that this antibody recognizes mCherry-Kv2.1-WT and Myc-Kv2.1-WT, but not the respective truncated (ΔC318) mutants. This blot also confirms the cell-surface expression of mCherry- Kv2.1-WT.

C) INS 832/13 cells expressing Myc-Kv2.1-WT, Myc-Kv2.1-ΔC318, or Myc alone were immunostained with anti-Myc (red) and anti-Kv2.1 (green; which recognizes full-length and endogenous Kv2.1 but not the truncated mutant). A high degree of colocalization was observed between the truncated Kv2.1 and endogenous Kv2.1, suggesting the interaction of Myc-Kv2.1-ΔC318 with endogenous Kv2.1 channels. Scale bar is 10 μm.

D) Density of native Kv2.1 clusters, assessed by immunostaining with the anti-Kv2.1 antibody and imaged withTIRF microscopy, in INS 832/13 cells expressing Myc alone or Myc-Kv2.1-ΔC318 (n=21, 34 cells). **$p<0.01$

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

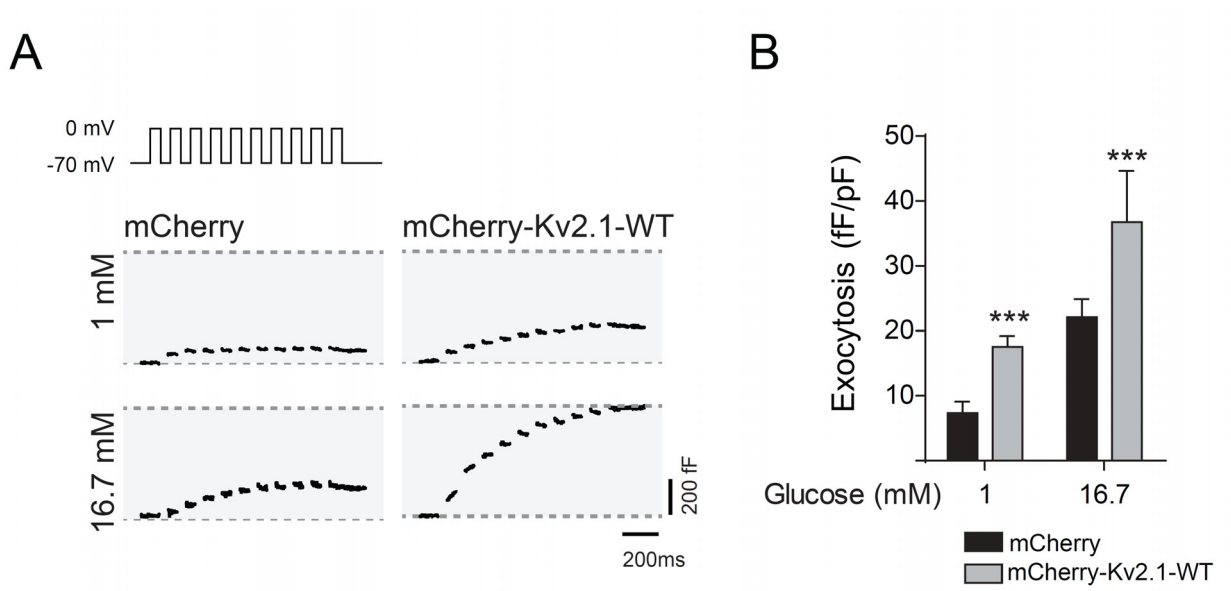
Supplementary Figure 6. Up-reguation of Kv2.1 ehances depolarization-induced exocytosis at low and high glucose in INS-1 832/13 cells.

Exocytosis was elicited by a series of 10 depolarizations from -70 to 0 mV and measured as increases in cell capacitance in INS-1 832/13 cells following transfection with a control plasmid (mCherry) or the mCherry-tagged wild-type Kv2.1 (mCherry-Kv2.1-WT). Experiments were performed after a 1 hour pre-incubation at 1 mM glucose and subsequent exposure of cells to 1 or 16.7 mM glucose in the patch-clamp bath solution.

A) Representative membrane capacitance responses.

C) Cumulative exocytotic responses over the series of 10 membrane depolarizations. n=26,36,25,17 cells.

***-p<0.001 compared with the mCherry groups.



SUPPLEMENTARY DATA

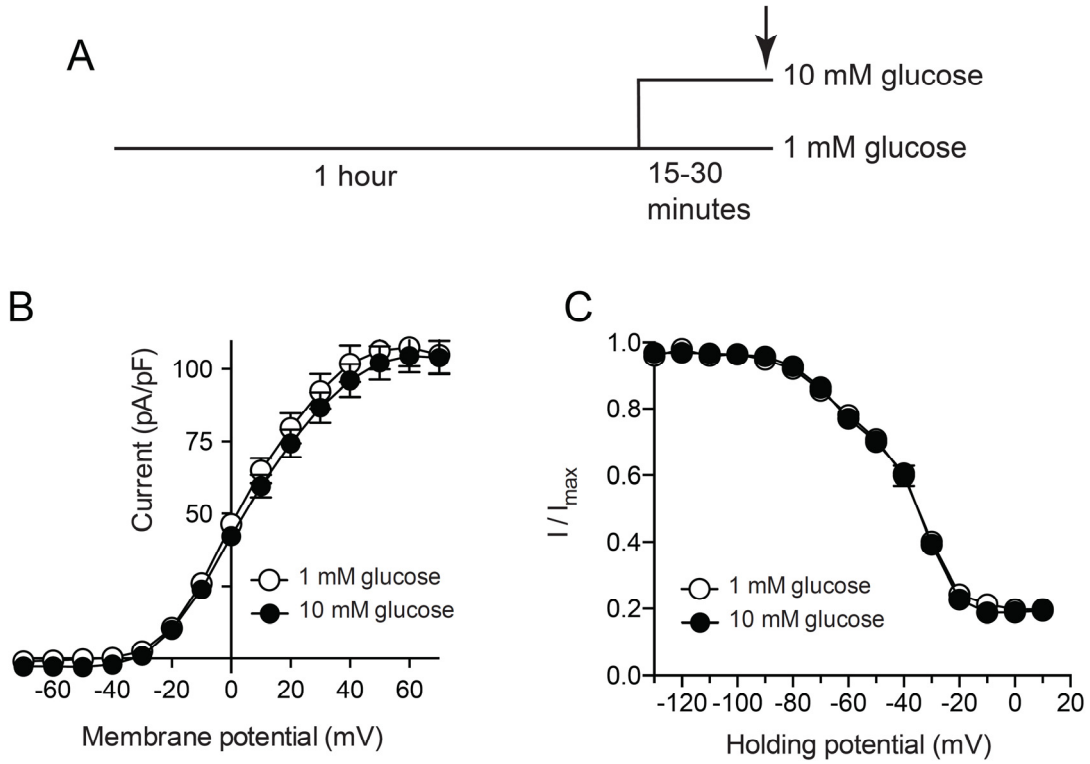
Supplementary Figure 7. No effect of prior glucose-stimulation on Kv currents recorded from human β-cells under the whole-cell patch-clamp condition.

Kv currents were recorded from human β-cells, positively identified by insulin immunostaining, under glucose-stimualtion conditions known to amplify the exocytotic resposne (Ferdaoussi et al, JCI, 2015).

A) Cells were pre-incubated at 1 mM glucose for 1 hour, and then exposed to 1 or 10 mM glucose in the bath solution for 15-30 minutes prior to making the whole-cell patch-clamp (at arrow) in order to test whether the resulting Kv currents were altered by glucose-treatment.

B) Current-voltage relationship of Kv currents elicted from a holding potential of -70 mV, measured from human β-cells (n=28 and 50 cells from 6 donors).

C) Voltage-dependence of steady-state inactivation of Kv currents from human β-cells from holding potentials ranging between -130 and 10 mV (n=35 and 40 cells from 6 donors).



SUPPLEMENTARY DATA

Supplementary Figure 8. Secretory dysfunction of islets from donors with T2D used in Figure 8J compared with matched controls.

Impaired insulin secretion was confirmed in the T2D islets (n=3 donors) used for rescue experiments in Figure 8J compared with non-diabetic donors (n=3 donors) matched for age (53.7+/-3.3 versus 51.7+/-2.8), sex (2F and 1M in each group), and BMI (28.6+/-3.8 versus 28.4+/-1.2).

A) Insulin content was lower in islets from T2D donors, although this did not reach statistical significance.

B) Insulin secretion, as a percentage of insulin content, in response to 16.7 mM glucose in static incubation experiments was reduced in T2D islets.

C) The stimulation index (fold change in insulin secretion from 1 to 16.7 mM glucose) was reduced in the T2D islets).

*-p<0.05 as indicated; **-p<0.01 compared with ND; and ***-p<0.001 compared with the ND 1 mM glucose.