SUPPLEMENTARY DATA

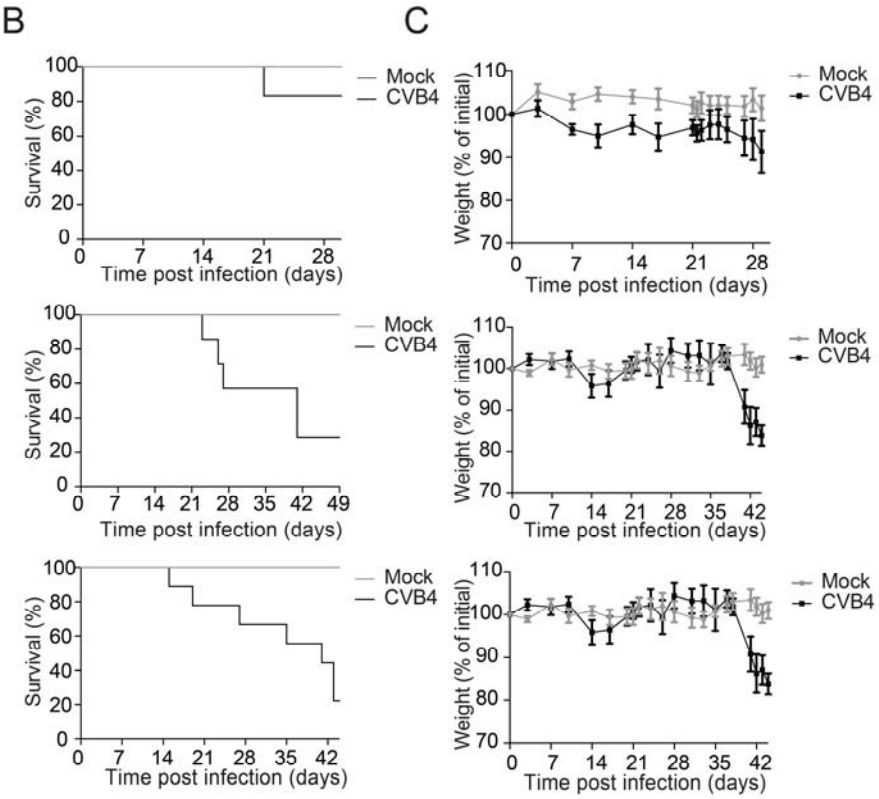
Supplementary Figure 1. Overview of human islet-engrafted mouse studies. (A) Summary of three experiments. **(B)** Survival of mice in each of the three experiments. Experiment 1: n = 5, mock-infected, n = 7, CVB4-infected. One infected, non-diabetic mouse required euthanasia at 21 dpi. Experiment 2: n = 7, mock-infected, n = 7, CVB4-infected. Three infected mice required euthanasia between 23 and 27 dpi due to severe fight wounds. Experiment 3: n = 5, mock-infected, n = 9, CVB4-infected. Four mice died prematurely between 15-35 dpi. **(C)** Average weight loss of mice in each of the three experiments. Body weight is expressed as the percent difference from the body weight of baseline. Experiment 1: n = 5, mock-infected, n = 7, CVB4-infected. Experiment 2: n = 7, mock-infected, n = 7, CVB4-infected. Experiment 3: n = 5, mock-infected, n = 9, CVB4-infected. For Experiment 1, only mice that survived beyond 21 dpi were included for further analysis. For Experiments 2 and 3, only mice that survived beyond 35 dpi were included for further analysis.

A

EXPERIMENT 1	
Mouse strain	NSG
Mouse β-cell disruption	STZ
Duration of study	30 days
Mock infected mice	n = 5

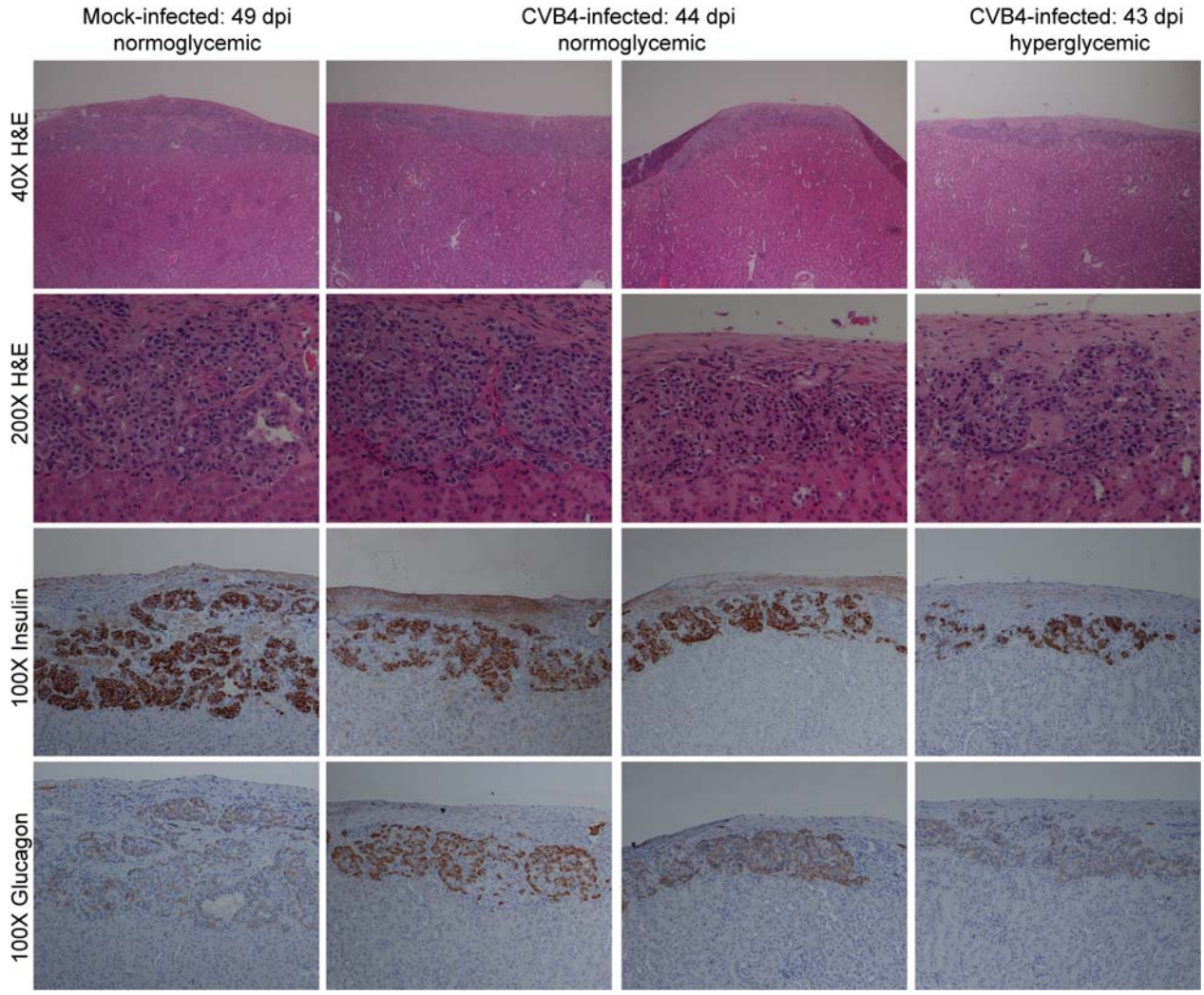
EXPERIMENT 2	
Mouse strain	NSG-Tg(RIP-DTR)
Mouse β-cell disruption	DTR
Duration of study	49 days
Mock infected mice	n = 7

EXPERIMENT 3	
Mouse strain	NSG-Tg(RIP-DTR)
Mouse β-cell disruption	DTR
Duration of study	44 days
Mock infected mice	n = 5



SUPPLEMENTARY DATA

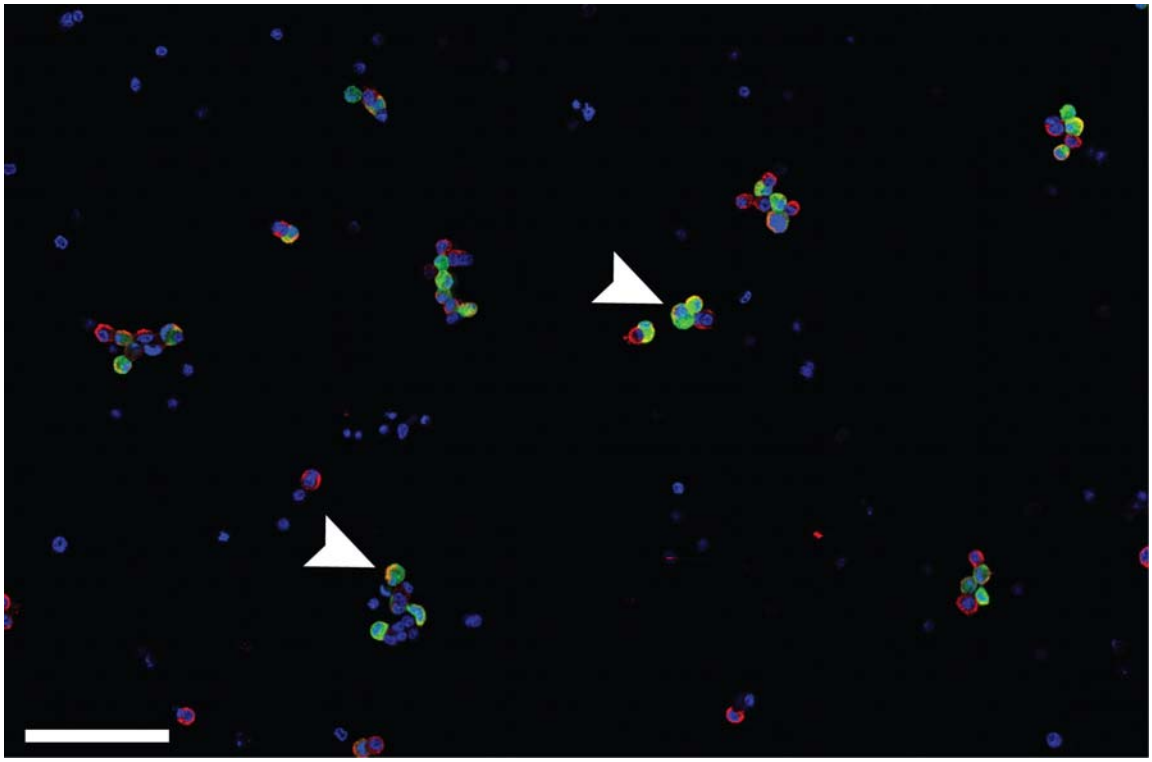
Supplementary Figure 2. Histology of human islet grafts from CVB4-infected mice, including a normoglycemic mouse and one that ultimately became diabetic. Histological sections from mice in Experiment 3 are shown here. H&E staining is shown at either 40x magnification (top row) or 200x magnification (second row). Immunohistochemical staining for either insulin (third row) or glucagon (bottom row) are also shown at 100x magnification. Findings in this experiment were similar to those described for Experiment 2 in Figure 2.



SUPPLEMENTARY DATA

Supplementary Figure 3. EGFP-CVB3 infects beta cells of cultured human islets. Dispersed human islets were infected with EGFP-CVB3 (1 x 10^6 PFU/100 IEQ). A subset of the virally infected cells is insulin positive by immunofluorescent staining (arrowheads). EGFP-CVB3 is green, insulin is red, DAPI is blue. Co-localization of CVB3 and insulin is yellow. Scale bar represents 75 μm.

Cultured islets were treated with TrypLE (Life Technologies) diluted 1:3 in PBS for 5 min at 37°C. Islet culture medium was added to stop the reaction, and cells were pelleted, resuspended in fresh medium, then allowed to attach to coverslips. Cells were cultured in the presence of virus or media control for 16 h, after which cells were washed, fixed with 3.7% paraformaldehyde, then permeabilized and blocked. Guinea pig antibody to insulin was added, followed by Alexa Fluor-594 goat antibody to guinea pig IgG. Coverslips were mounted with ProLong Gold Antifade Reagent with DAPI. Confocal images were acquired using a Leica TCS SP8 DNI6000 confocal microscope using Leica image acquisition software LAS AF Version 3.1.0 build 8578.



SUPPLEMENTARY DATA

Supplementary Table 1. Demographic characteristics of human islet donors

	Donor 1	Donor 2	Donor 3
Age, years	55	55	29
Gender (M/F)	M	F	M
Ethnicity	n.r.	White	Hispanic/Latino
Body weight, kg	85.0	109.1	87.3
BMI, kg/m^2	28.4	39.9	27.5
Time in culture*	16 h	28 h	20 h
HLA	n.r.	n.r.	Class 1 – A: 2, 11 Class 1 – B: 7, 51 Class 1 – C: 7, 15 Class 2 – DR: 8, 15

Abbreviations: BMI, body mass index; n.r., not recorded. *Refers to the amount of time that the human islets were cultured following isolation until shipment to our laboratory.

SUPPLEMENTARY DATA

Supplementary Table 2. Normalized gene copy numbers in islet grafts for genes with significant increases or decreases in expression (CVB4-infected versus mock-infected) when data from three experiments were combined; mean values are shown for each experiment.

	Experiment 1				Experiment 2				Experiment 3			
Genes with increased expression	**Mock Average (n=5)**	**CVB4 Average (n=6)**	***P* value**		**Mock Average (n=5)**	**CVB4 Average (n=4)**	***P* value**		**Mock Average (n=5)**	**CVB4 Average (n=4)**	***P* value**	
CXCL10	7	32	0.035	*	4	23	0.114	ns	6	27	0.020	*
MX1	55	236	0.004	**	71	151	0.378	ns	54	140	0.019	*
HLA-A	170	515	0.001	**	229	447	0.060	ns	180	370	0.048	*
OAS2	5	15	0.020	*	5	17	0.331	ns	8	21	0.006	*
TXNIP	271	493	0.022	*	193	402	0.050	*	204	585	0.057	ns
CCL5	5	9	0.233	ns	3	9	0.088	ns	4	12	0.299	ns
IFIH1	13	28	0.004	**	11	18	0.022	*	7	15	0.009	*
CASP1	7	15	0.005	*	3	8	0.008	*	6	9	0.318	ns
CCL3	1	3	0.090	ns	1	3	0.273	ns	3	6	0.230	ns
IL10	2	2	0.926	ns	1	5	0.060	ns	2	3	0.170	ns
TNFAIP3	38	41	0.575	ns	25	47	0.157	ns	29	87	0.128	ns
DDX58	208	315	0.002	**	148	239	0.198	ns	140	251	0.078	ns
SIRPG	4	5	0.227	ns	1	6	0.080	ns	4	6	0.650	ns
DDIT3	326	437	0.039	*	164	250	0.025	*	141	211	0.008	*
IRF1	24	36	0.013	*	17	22	0.344	ns	20	30	0.148	ns
STAT1	39	81	0.012	*	41	47	0.804	ns	36	46	0.337	ns
CCDC88A	17	20	0.300	ns	12	30	0.050	*	18	21	0.711	ns
NMI	6	7	0.227	ns	3	3	0.694	ns	3	6	0.141	ns
EIFAK3	120	162	0.001	**	83	96	0.457	ns	85	123	0.013	*
GSK3B	280	313	0.101	ns	254	320	0.164	ns	212	263	0.049	*
Genes with decreased expression												
INS	38456	15143	0.004	**	17383	5410	0.011	*	11617	3977	0.003	**
PDX1	122	62	0.022	**	92	26	0.023	*	55	20	0.009	*
SST	5848	3060	0.047	*	5144	1201	0.005	*	1925	920	0.007	*
IL1B	35	18	0.012	*	17	13	0.362	ns	17	6	0.064	ns
WFS1	56	42	0.047	*	57	15	0.008	*	33	18	0.002	**
XBP1	129	93	0.176	ns	99	36	0.043	*	48	29	0.041	*
SREBF1	101	85	0.353	ns	123	44	0.028	*	59	39	0.054	ns
CTLA4	34	16	0.0005	***	16	14	0.679	ns	15	11	0.294	ns

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant, Student's t-test

SUPPLEMENTARY DATA

Supplementary Table 3. Mean normalized gene copy numbers in *ex vivo* cultured islets for genes with >2.5 fold increases or decreases in expression (CVB4-infected versus mock-infected).

Genes with increased expression	Mock Average (n=3)	CVB4 Average (n=3)	*P* value	
CXCL10	5	217	0.080	ns
OAS2	3	71	0.007	*
CCL3	2	35	0.040	*
IFNG	3	39	0.005	*
CREB3L3	3	39	0.117	ns
MX1	54	641	0.004	*
CCL4	6	46	0.121	ns
CCL5	6	41	0.048	*
IFNB1	8	63	0.019	*
HNF1A	5	33	0.047	*
TLR3	6	28	0.009	*
EIF2AK3	14	56	0.062	ns
HMOX1	29	136	0.031	*
GK	16	67	0.002	*
IL8	1159	4784	0.044	*
NMI	8	31	0.039	*
KCNQ1	16	60	0.119	ns
PDE4B	6	22	0.073	ns
PTPN22	2	6	0.153	ns
SKAP2	14	40	0.004	*
IFIH1	10	32	0.141	ns
STAT1	29	91	0.136	ns
CASP8	10	29	0.004	*
CCDC88A	8	28	0.325	ns
TCF7L2	30	83	0.102	ns
Genes with decreased expression				
INS	17388	2764	0.034	*
SST	7331	1192	0.071	ns
PDX1	152	34	0.056	ns
CTSH	175	54	0.030	*
CTRB2	527	200	0.066	ns
CTLA4	20	7	0.117	ns

*, $P < 0.05$; ns, not significant, Student's t-test

A Supplementary Spreadsheet is also available. This includes sequences for the NanoString human gene CodeSet, normalized gene expression values from grafts from *in vivo* and *ex vivo* experiments, and body weights and glucose values for all mice.