Online Supplemental Materials for:

Coxsackievirus B vaccines prevent infection-accelerated diabetes in NOD mice and have no disease inducing effect

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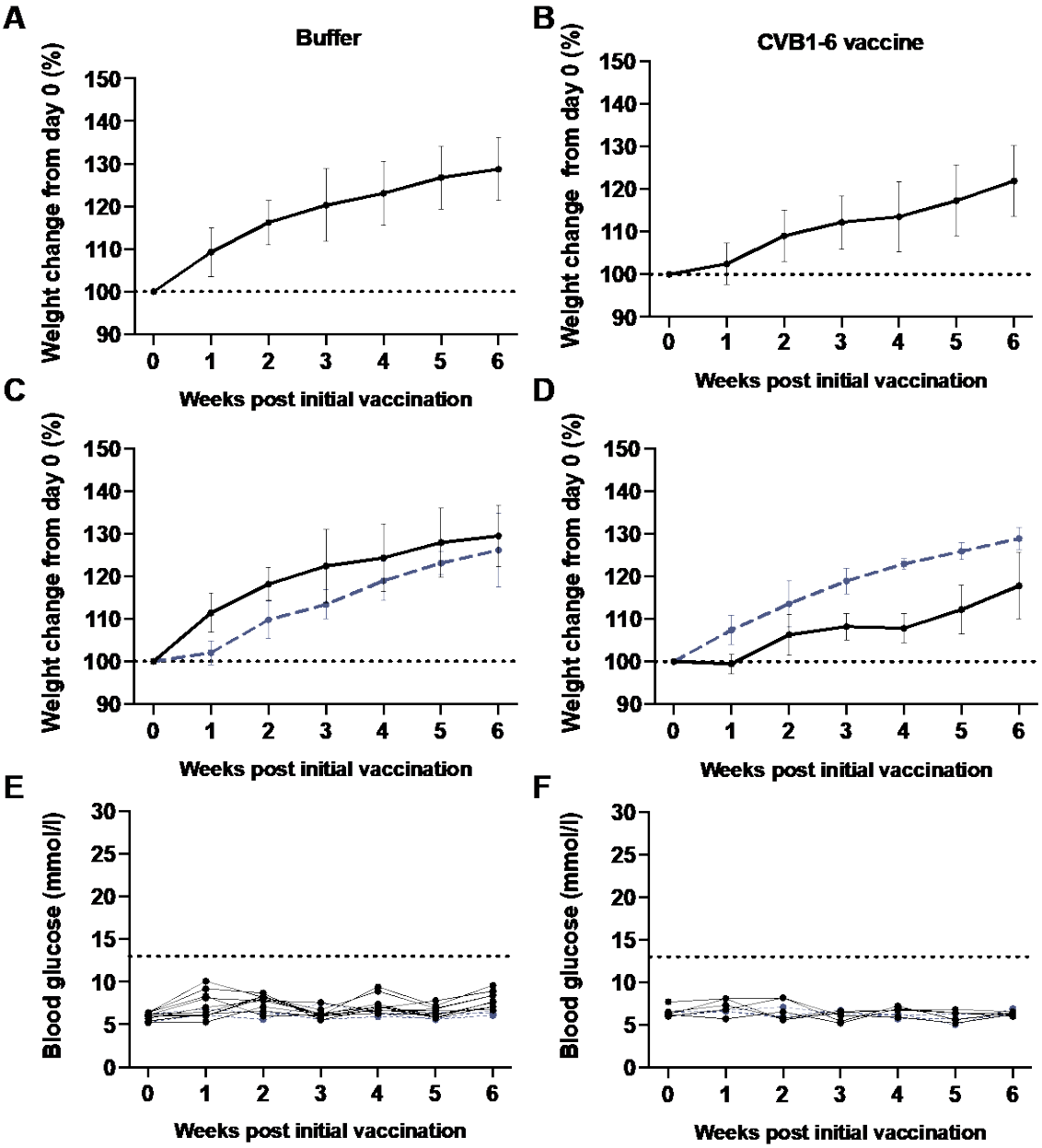
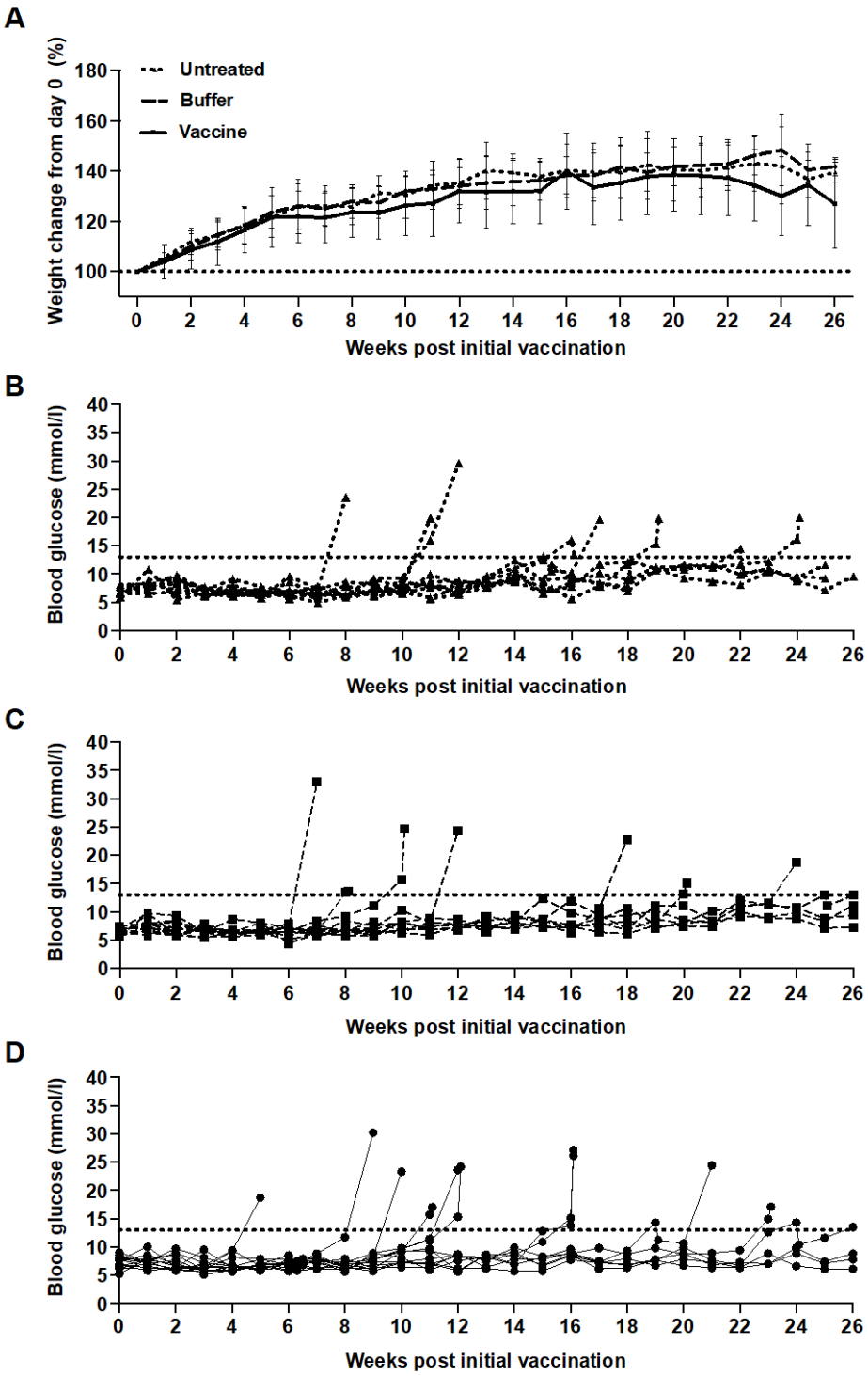
Supplementary Table 1: Reagents and suppliers

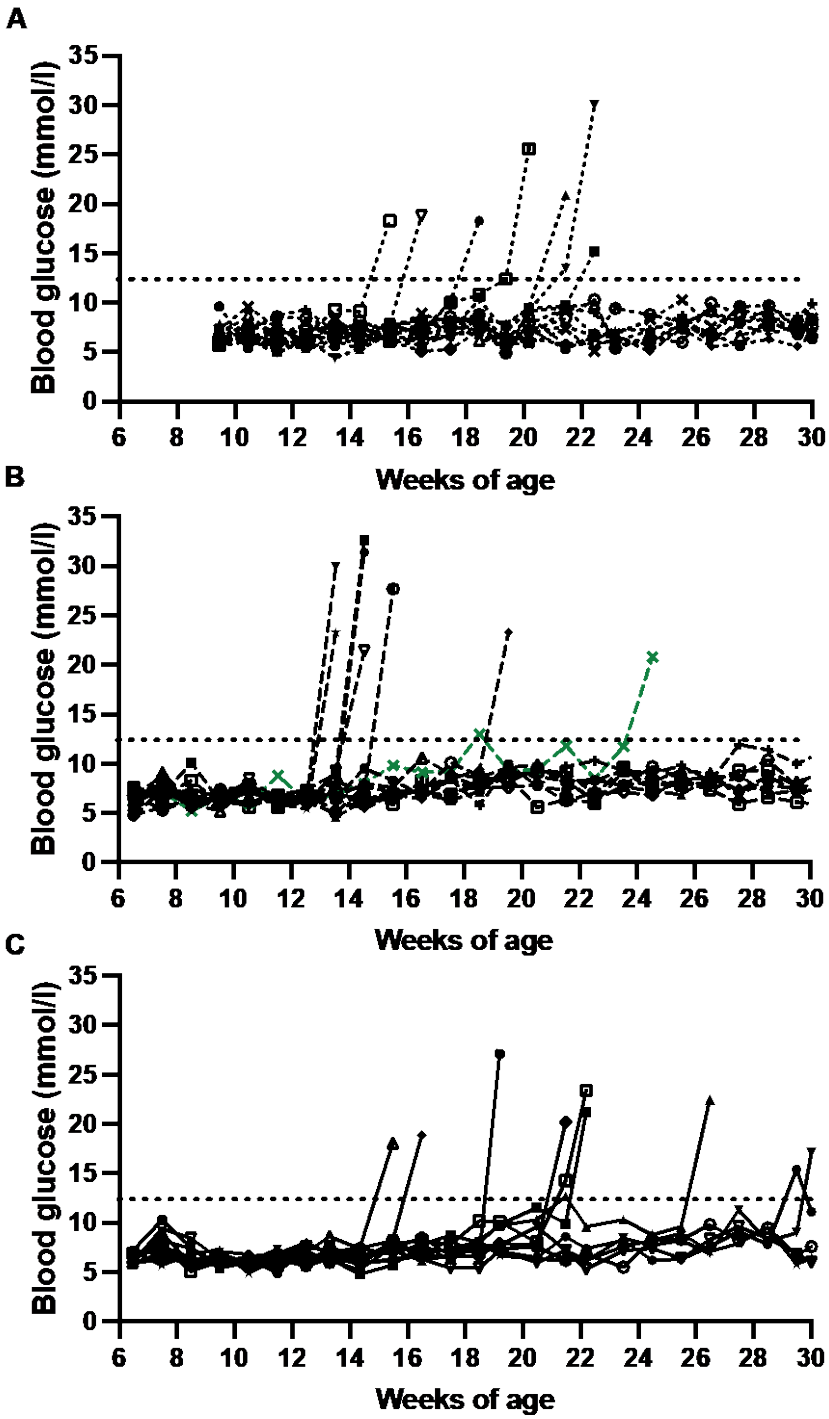
REAGENT or RESOURCE	SOURCE	REFERENCE
Antibodies		
Guinea pig anti-insulin 1:20,000	DakoCytomation	A0564, N1542
Rabbit anti-Glucagon 1:12000	Abcam	EP3070, Ab92517
Goat anti-guinea pig 1:200	Vector Laboratories	W0762, BA-7000
Goat anti-rabbit 1:200	Dako	E0432
Biological Samples		
Formalin fixed paraffin embedded mouse pancreas		
Chemicals, Peptides, and Recombinant Proteins		
M199 Medium	Gibco	11043-023
Carboxymethylcellulose	Sigma-Aldrich	C5013
Immunohistochemistry PAP pen	Dako	S2002
Normal Goat Serum (used concentrations 10% and 2%)	Dako	X0907
Elite ABC HRP Detection Kit	Vectastain	PK-6100
DAB Peroxidase Substrate Kit	Vector	SK-4100
Hematoxylin Mayer's	Sigma-Aldrich	MHS32



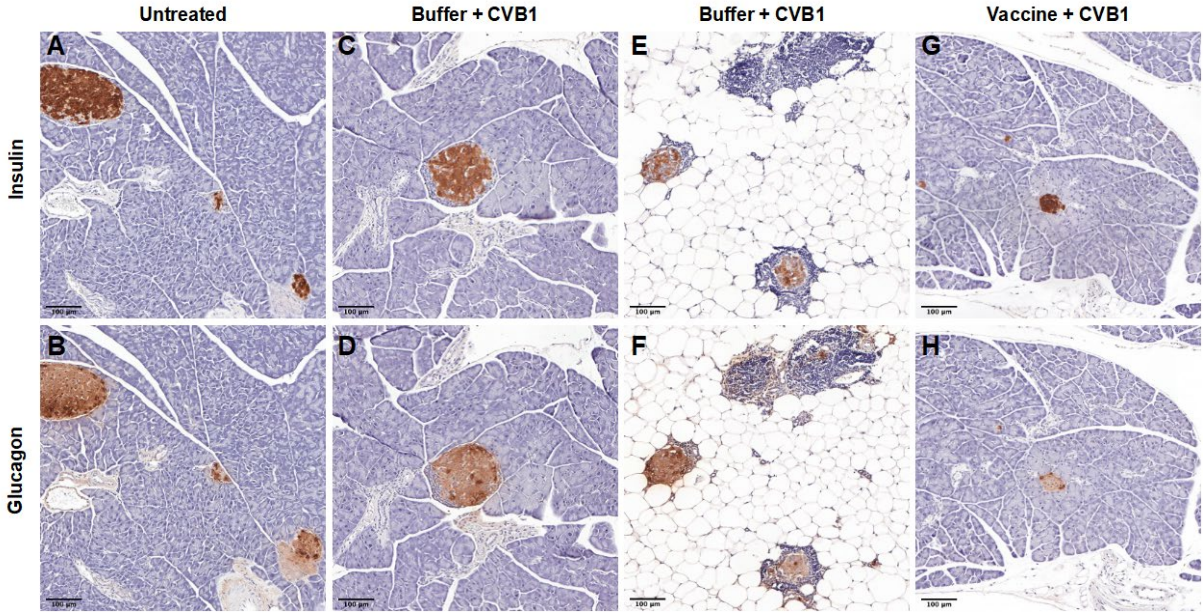
Supplementary Figure 1: CVB1-6 vaccine has no adverse effects on weight or blood glucose. Female NOD mice (5.1 - 6.3 weeks old) were mock vaccinated (buffer, n=13) or vaccinated with CVB1-6 vaccine (n=8) by interscapular (subcutaneous) injection on three occasions (on days 0, 14 and 28, n=3 or on days 0, 21 and 35, n=5). (a, b) Percentage weight change from day 0 in buffer treated (left) and CVB1-6 vaccinated mice (right). Shown are the mean values ± SD. The dotted line indicates the weight prior to the first vaccination on day 0. In (b) the weight data has been separated into new data (black lines) and data previously published in (1). (c) Blood glucose values for the buffer treated (left) and CVB1-6 vaccinated (right) mice from day 0. The dotted line indicates the diabetes threshold. The blue lines were previously published in (1).



Supplementary Figure 2: CVB1-6 vaccine has no adverse effects on weight or blood glucose. Female NOD mice (4.9 - 7.1 weeks old) were left untreated (n=10), mock-vaccinated (n=15) or vaccinated (n=14) with CVB1-6 vaccine by interscapular (subcutaneous) injection on either two (days 0 and 21; n=6 for buffer, n=10 for CVB1-6 vaccine) or three (days 0, 21 and 35; n=9 for buffer, n=4 for CVB1-6 vaccine) occasions. (a) Percentage weight change from day 0 in untreated, mock-vaccinated (buffer) and CVB1-6 vaccinated mice. Shown are the mean values ± SD. The dotted line indicates the weight prior to the first vaccination on day 0. (b) Blood glucose values for the untreated (b), mock-vaccinated (buffer) (c) and CVB1-6 vaccinated (d) mice from day 0 post initial vaccination. The dotted line indicates the diabetes threshold.



Supplementary Figure 3: CVB1 vaccine protects against CVB1 accelerated diabetes. Female NOD mice were left (a) untreated (dotted lines; n=16; blood glucose levels monitored from 8 weeks of age), (b) mock-vaccinated with vaccine buffer and infected with CVB1 virus (dashed lines; buffer + CVB1; n=16; 6.3 – 6.9 weeks old) or (c) vaccinated with CVB1 vaccine and infected with CVB1 virus (solid lines; vaccine + CVB1; n=12; 6.3 – 6.9 weeks old). Vaccinations (buffer or vaccine injections) were performed on days 0, 21 and 35 and the mice were infected with virus (10^7 PFU by i.p. injection, total volume 200µl) on day 42 (12.3 -12.9 weeks of age). (a-c) Blood glucose levels were monitored up to 30 weeks of age. The dotted line indicates the diabetes threshold. In (b) the mouse in green was borderline diabetic until 25 weeks of age and excluded from some of the statistical analyses performed in Fig. 3e in the main article text.



Supplementary Figure 4: CVB1 vaccine prevents CVB1-mediated exocrine tissue destruction. Female NOD mice were left untreated (a, b; n=15; blood glucose levels monitored from 8 weeks of age), mock-vaccinated with vaccine buffer and infected with CVB1 virus (c-f; buffer + CVB1; n=16; 6.3 – 6.9 weeks old) or vaccinated with CVB1 vaccine and infected with CVB1 virus (g, h; vaccine + CVB1; n=12; 6.3 – 6.9 weeks old). Vaccinations (buffer or vaccine injections) were performed on days 0, 21 and 35 and the mice were infected with virus (10^7 PFU by i.p. injection, total volume 200µl) on day 42 (12.3 -12.9 weeks of age). Mice were followed until diabetes onset or 30 weeks of age and at the terminal timepoints pancreas was collected for histological analysis. (a-h) Representative images of pancreas histology from mice that did not develop diabetes by the terminal endpoint. Sequential sections were stained with insulin (a, c, e, g) or glucagon (b, d, f, h) and assessed by light microscopy. The images in c-f come from the same mouse and show a part of the exocrine tissue with healthy appearance (c, d) and another part with extensive fatty replacement of acinar cells by fat (e, f). Scale bars are shown in the bottom left-hand corner of each image (scale bar = 100µm).

References

1. Stone VM, Hankaniemi MM, Laitinen OH, Sioofy-Khojine AB, Lin A, Diaz Lozano IM, Mazur MA, Marjomaki V, Lore K, Hyoty H, Hytonen VP, Flodstrom-Tullberg M: A hexavalent Coxsackievirus B vaccine is highly immunogenic and has a strong protective capacity in mice and nonhuman primates. Sci Adv 2020;6:eaaz2433