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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: VP. Performed the experiments: VP, DF, HRD, LIB, BHE, AKFM. Analyzed the data: VP, HRD. Wrote the paper: VP, PS.

SUPPLEMENTAL METHODS

Vaccination and CVB3 Infection of Mice. 4-week-old p48^{cre};LSL-*Kras*^{G12D} (KC) mice (detailed info in (1)) and their non-transgenic littermates were used for in vivo testing of the CVB3 peptide vaccines. Two linear peptides (HPLC purity > 98%) from the CVB3 capsid protein, VP1-1: GPVEDAITAAIGRVA(C) and VP1-24: IKSTIRIYFKPKHVK(C), were conjugated to KLH at the added cysteine residue to increase the immunogenicity of the peptides in vivo (GenScript, Piscataway, NJ). A mixture of both peptides was used for vaccination. 50 µg of each peptide was mixed with Complete Freund's Adjuvant (Invivogen, San Diego, CA) for the first dose and Incomplete Freund's Adjuvant (Invivogen) for the subsequent five booster doses in a 1:1 ratio and injected subcutaneously into the mice. The vehicle control was adjuvant alone. The schedule and frequency of vaccination are depicted in **Figure 1A**. After a total of 6 doses of vaccine over a period of 4 weeks, mice were infected with CVB3 (6.22 x 10^4 PFU/0.1 ml) in a volume of 50 µl (VR-30, ATCC) intraperitoneally. 6 weeks post infection, mice were euthanized and tissue was collected for analysis.

Mouse Lines and Sex as a Biological Variable. LSL-*Kras*^{G12D} mice were obtained from the NCI Mouse Repository (MMHCC). The p48^{Cre} mice were a gift from Dr. Pinku Mukherjee, University of North Carolina. Both lines were crossed to generate bi-transgenic p48^{Cre};LSL-*Kras*^{G12D} (KC) mice with C57BL/6J background. Since we did not observe sex differences in KC mice after CVB3 infection in a similar experimental setting in our previous work (1), sex was not considered as a biological variable.

Blood Collection. During the 4-week period of vaccination and at the termination of the experiment, blood was collected from mice at indicated time points (**Fig. 1A**) via submandibular bleeding using 4 mm lancets (Braintree Scientific Inc., Braintree, MA). Approximately 100 µl blood was collected in serum separator gel microtainer tubes (BD Biosciences, Franklin Lakes, NJ) and incubated on ice for 30 minutes. Tubes were centrifuged at 10,000 g at 4 °C for 20 minutes to separate the serum from other blood components. Serum was carefully pipetted out and transferred to fresh tubes for analysis via ELISA.

ELISA. For the ELISA to detect CVB3 antibodies in mouse sera, the VP1-1 peptide was diluted to 10 ng/µl in a coating buffer (50 mM carbonate/bicarbonate; pH 9.6). Immulon 2HB flat-bottom microtiter plates (ThermoFisher Scientific, Waltham, MA) were coated with 0.5 µg of the peptide. Mouse IgG (Sigma Aldrich, St. Louis, MO) standard dilutions were prepared (500 ng/ml to 0.48 ng/ml). Controls including diluted peptide, IgG standards and coating buffer were added, and the plate was covered and incubated (4 °C, overnight). Next day, contents of the plate were removed, and the plate was washed with 200 µl wash buffer (1X PBS, 0,05% Tween-20) for 3 times, 5 minutes each. All wells were then blocked with 200 µl blocking buffer (1% BSA in PBS) for 2 hours at RT while rocking gently. After a brief wash, diluted serum samples were added, and blocking buffer was added to the wells with IgG standards. The plate was covered and incubated at 4 °C, overnight, then 3 times washed with wash buffer. HRP-conjugated secondary antibody (Millipore, Burlington, MA) was added to all the wells at a dilution of 1:2000 for 1 hour at

RT. The plate was washed and developed by adding TMB substrate solution (ThermoFisher Scientific) for 1 min while shaking. The reaction was stopped with 1N HCl solution per well, and the absorbance was determined at 450 nm wavelength on a microplate reader (BioTek Synergy HT, Agilent technologies, Santa Clara, CA).

Antibodies. Antibodies for CD3 (ab11089), CD4 (ab183685), CD8 (ab209775), or smooth muscle actin (SMA) (ab5694) were from Abcam (Cambridge, MA). Antibodies for F4/80 (MCA497R) and Ly6B.2 (MCA771G) were from AbD Serotech (Raleigh, NC), antibodies for FoxP3 (12653) from Cell Signaling (Danvers, MA) and for Ym1 (60130) from STEMCELL Technologies (Vancouver, Canada). The rabbit anti-CVB3 capsid antibody (used in **Fig. 1C**) is custom made and was generated by 21st Century Biochemicals (Marlborough, MA), with C-PEG4-GPVEDAITAAIGRVA-amide as an immunogen. Initial bleeds were tested via Western blot for immunoreactivity towards the CVB3 capsid. Positive bleeds were then pooled and affinity purified using peptide columns. Purified antibodies were verified for specificity via Western blot analyses and by comparing IHC staining to *in situ* hybridization (ISH) for presence of CVB3 RNA (not shown).

Immunohistochemistry (IHC), Immunofluorescence (IF)-IHC, H&E and Trichrome Staining. Samples were deparaffinized (1 hr, 60 °C), de-waxed in xylene (five times, 4 min) and re-hydrated with ethanol (100%, 95%, 75% - each two times, 3 min). Samples were then rinsed with water and subjected to antigen retrieval (citrate buffer pH 6.0).

Samples were treated with 3% hydrogen peroxide (5 min), washed with PBS 0.5% Tween 20, and incubated with protein block serum-free solution (DAKO) (5 min, RT). Dilution of primary antibodies are indicated in **Supplemental Table 1**. For IHC, primary antibodies were diluted in Antibody Diluent Background Reducing Solution (DAKO) and visualized using the EnVision Plus Anti-Rabbit Labelled Polymer Kit (DAKO). For immunofluorescence on tissue (IF-IHC), primary antibodies were diluted in 150 µl Antibody Diluent (DAKO) per slide. Slides were washed (PBS/0.5% Tween20) three times and incubated with Alexa-Fluor labeled secondary antibodies (Invitrogen, Carlsbad, CA) at 1:500 and DAPI (PeproTech, Rocky Hill, NJ) at 125 µg/ml for 1 hr, and washed again for 3 times. Antibodies used, and a more detailed procedure, are described in (2). For trichrome staining, tissue slides were stained using Masson Trichrome Stain Kit (Sigma-Aldrich). H&E staining is described in (1). Images (IHC, Trichrome, H&E, IF-IHC) were captured using the Aperio AT2 or the Aperio Fluorescence scanners (Leica Biosystems, Buffalo Grove, IL), and analyzed with ImageScope software.

Reproducibility. Mice were randomly assigned with approximately equal numbers of males and females in each treatment group. Sample size was based on our previous study (1). Investigators were not blinded to allocation during experiments and outcome assessment. No data was excluded. The mouse model usually doesn't show high variations at the time points of analyses (1) and n=3-7 mice per sample group were efficient to observe highly-statistically significant effects. Immunohistochemistry was quantified by the Aperio Positive Pixel Count Algorithm or by manual counting of positive cells.

Statistics. Data in bar graphs are presented as mean values. All error bars represent ± standard deviation (SD). Statistical analyses were performed with GraphPad Prism (GraphPad Inc., La Jolla, CA) and statistical significance between groups was determined using a two-tailed t-test. $p<0.05$ was considered statistically significant.

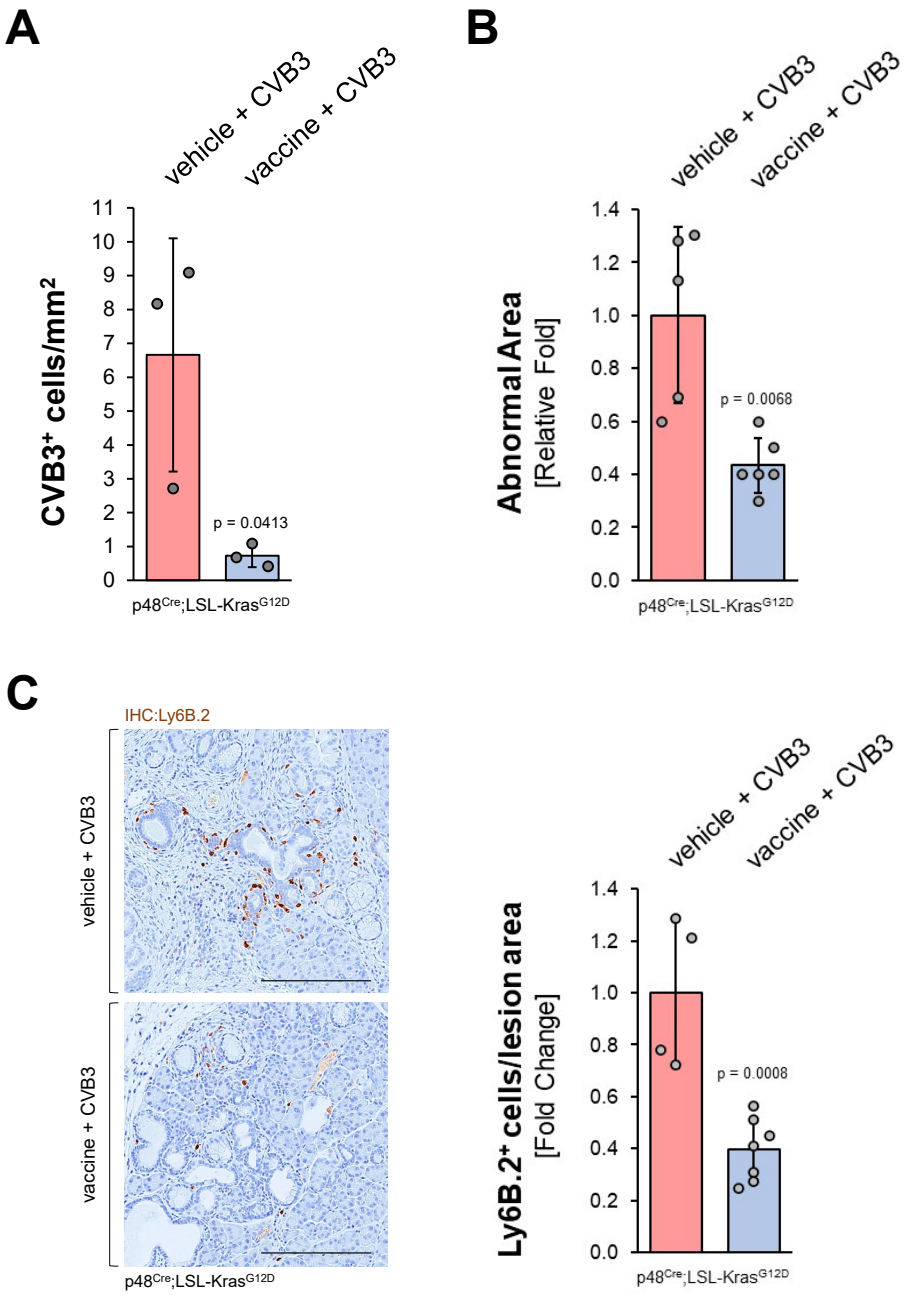
Study Approval. The animal experiments were approved by the Mayo Clinic (Rochester, MN) Institutional Animal Care and Use Committee (IACUC) under protocol A00001701-16-R19.

Data Availability. Values for all data points in graphs are available in the Supporting Data Values file.

References for Experimental Procedures

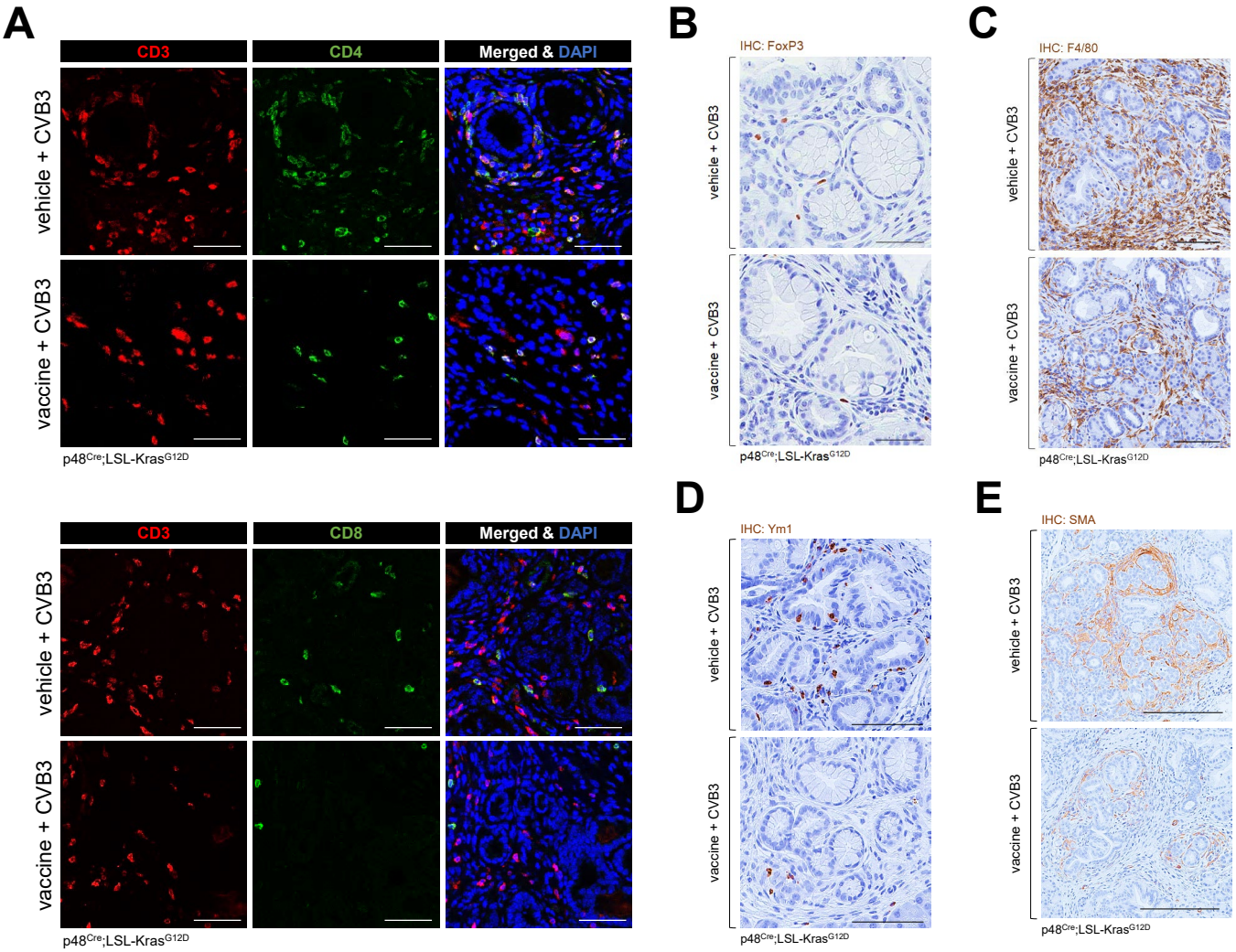
1. Bastea LI, et al. Coxsackievirus and adenovirus receptor expression facilitates enteroviral infections to drive the development of pancreatic cancer. *Nat Commun.* 2024;15(1):10547.
2. Pandey V, et al. CXCL10/CXCR3 signaling contributes to an inflammatory microenvironment and its blockade enhances progression of murine pancreatic precancerous lesions. *Elife.* 2021;10:e60646.

Supplemental Figure S1



Supplemental Figure S1. A: Corresponding to Figure 1C. Pancreatic tissues (n=3 per experimental group) of CVB3 infected KC mice either previously vehicle treated or vaccinated were quantified for CVB3$^+$ cells. Statistical significance between two sample conditions was determined using the t-test and statistical significance is indicated by a p value < 0.05. **B: Corresponding to Figure 1D.** Pancreatic tissues (individual mice indicated by dots) of CVB3 infected KC mice either previously vehicle treated or vaccinated were quantified for abnormal tissue area. Statistical significance between two sample conditions was determined using the t-test and statistical significance is indicated by a p value < 0.05. **C: Analysis of presence of neutrophils.** Pancreatic tissues of CVB3 infected KC mice either previously vehicle treated or vaccinated were analyzed by IHC for presence of neutrophils using Ly6B.2 as a marker. The pictures show a representative lesion area. The bar indicates 200 µm. The bar graph shows a quantification (individual mice indicated by dots). Statistical significance between two sample conditions was determined using the t-test and statistical significance is indicated by a p value < 0.05.

Supplemental Figure S2



Supplemental Figure S2. A: Corresponding to Figure 1F. Pancreatic tissues of CVB3 infected KC mice either previously vehicle treated or vaccinated were analyzed by IF-IHC for presence of $CD3^+;CD4^+$ (top panel) or $CD3^+;CD8^+$ (bottom panel) T cells. The merged picture includes a DAPI staining. The pictures a representative lesion area. The bar indicates 50 µm. **B: Corresponding to Figure 1G.** Pancreatic tissues of CVB3 infected KC mice either previously vehicle treated or vaccinated were analyzed by IHC for presence of Treg using FoxP3 as a marker. The pictures show a representative lesion area. The bar indicates 50 µm. **C: Corresponding to Figure 1H.** Pancreatic tissues of CVB3 infected KC mice either previously vehicle treated or vaccinated were analyzed by IHC for presence of macrophages using F4/80 as a marker. The pictures show a representative lesion area. The bar indicates 100 µm. **D: Corresponding to Figure 1I.** Pancreatic tissues of CVB3 infected KC mice either previously vehicle treated or vaccinated were analyzed by IHC for presence of AAM using Ym1 as a marker. The pictures show a representative lesion area. The bar indicates 200 µm. **E: Corresponding to Figure 1J.** Pancreatic tissues of CVB3 infected KC mice either previously vehicle treated or vaccinated were analyzed by IHC for presence of SMA^+ cells (activated stellate cells). The pictures show a representative lesion area. The bar indicates 200 µm.

Supplemental Table 1

Antibody	Company/Source	Catalog Number	Species	IHC	IF-IHC
CVB3	Storz Laboratory		rabbit	1:100	
CD3	Abcam	ab11089	rat		1:200
CD4	Abcam	ab183685	rabbit		1:1000
CD8	Abcam	ab209775	rabbit		1:500
F4/80	AbD Serotec	MCA497R	rat	1:200	
FoxP3	Cell Signaling	12653	rabbit	1:400	
Ly6B.2	AbD Serotec	MCA771G	rat	1:2000	
Ym1	STEMCELL Technologies	60130	rabbit	1:200	
SMA	Abcam	ab5694	rabbit	1:200	

Supplemental Table 1. **Antibodies and Dilutions.** Antibodies used were from the following sources: Abcam (Cambridge, MA), AbD Serotech (Raleigh, NC), Cell Signaling (Danvers, MA), and STEMCELL Technologies (Vancouver, Canada).